THE ANALYST

PROCEEDINGS OF THE SOCIETY FOR ANALYTICAL CHEMISTRY

NEW MEMBERS

Ordinary Members

Gerald Gordon Amery, A.R.I.C., A.F.Inst.Pet.; Arthur Churchman, M.Sc., F.R.I.C.; Michael Wyndham George Coldham, M.A., B.Sc. (Oxon.); Ronald St. John Emery; George Alexander Gray; Rodrigo Alberto Guedes de Carvalho, Dr.Chem.Eng. (Porto); John Jordan; Harold Pugh, M.Sc., A.R.I.C.; Arthur Derek Rudin, B.Sc. (Birm.); Gaston Albert Stroud; Murray M. Tuckerman, B.S. (Yale), B.S. (Temple), Ph.D. (R.P.I.).

Junior Member

Benjamin Chukuma Ndubisi Madu.

DEATH

We record with regret the death of

Charles Lessner.

NORTH OF ENGLAND SECTION

An Ordinary Meeting of the Section was held at 2.15 p.m. on Saturday, December 5th, 1959, at the City Laboratories, Mount Pleasant, Liverpool, 3. The Chair was taken by the Chairman of the Section, Dr. J. R. Edisbury.

The following paper was presented and discussed: "The Analysis of Cocoa and Chocolate in Relation to Modern Manufacturing Methods," by B. W. E. Minifie, F.R.I.C., and C. Harris, F.R.I.C.

SCOTTISH SECTION

A Joint Meeting of the Scottish Section and the Glasgow and West of Scotland Section of the Royal Institute of Chemistry was held at 7.15 p.m. on Friday, November 13th, 1959, in the Royal College of Science and Technology, Glasgow. The Chair was taken by the Chairman of the Scottish Section, Mr. A. N. Harrow, A.H.-W.C., F.R.I.C.

The following paper was presented and discussed: "Techniques in Radiochemistry for Analysis and Research," by D. A. Lambie, B.Sc., F.R.I.C.

WESTERN SECTION

A Joint Meeting of the Western Section with the Cardiff and District Section of the Royal Institute of Chemistry and the South Wales Section of the Society of Chemical Industry was held at 7 p.m. on Friday, December 11th, 1959, in the University College, Cardiff. The Chair was taken by the Chairman of the Western Section, Mr. S. Dixon, M.Sc., F.R.I.C.

The following paper was presented and discussed: "The Work of the Railway Chemist," by E. D. Henley, B.Sc., F.R.I.C.

MIDLANDS SECTION

An Ordinary Meeting of the Section was held at 6.30 p.m. on Thursday, November 12th, 1959, in the Mason Theatre, The University, Edmund Street, Birmingham, 3. The Chair was taken by the Chairman of the Section, Dr. S. H. Jenkins, F.R.I.C., F.Inst.S.P.

The following paper was presented and discussed: "The Identification and Determination of Phenols," by L. Barker, B.Sc., Ph.D., A.R.I.C.

An Ordinary Meeting of the Section was held at 7 p.m. on Wednesday, December 9th, 1959, at the Wolverhampton and Staffordshire College of Technology, Wulfruna Street, Wolverhampton. The Chair was taken by the Chairman of the Section, Dr. S. H. Jenkins, F.R.I.C., F.Inst.S.P.

A discussion on "The Determination of Trace Impurities in Metals" was opened by B. Bagshawe, A.Met., and W. T. Elwell, F.R.I.C.

MICROCHEMISTRY GROUP

The twenty-second London Discussion Meeting of the Group was held at 6.30 p.m. on Wednesday, December 16th, 1959, in the restaurant room of "The Feathers," Tudor Street, London, E.C.4. The Chair was taken by the Vice-Chairman of the Group, Mr. C. Whalley, B.Sc., F.R.I.C.

A discussion on "Applications of the Flask Combustion" was opened by M. Corner, B.Sc., F.R.I.C., and C. B. Dennis.

PHYSICAL METHODS GROUP

The fifteenth Annual General Meeting of the Group was held at 6.30 p.m. on Tuesday, November 24th, 1959, in the meeting room of the Chemical Society, Burlington House, London, W.1. The Chair was taken by the Chairman of the Group, Mr. R. A. C. Isbell, A.Inst.P. The following appointments were made for the ensuing year:—*Chairman*—Dr. G. W. C. Milner. *Vice-Chairman*—Dr. W. Cule-Davies. *Hon. Secretary and Treasurer*—Dr. T. L. Parkinson, Product Research Division, Beecham Foods Ltd., Beecham House, Great West Road, Brentford, Middlesex. *Members of Committee*—Messrs. J. Allen, J. H. Glover, C. A. Parker, J. W. Price, G. F. Reynolds and J. Sanders. Dr. D. C. Garratt and Mr. C. A. Bassett were re-appointed as Honorary Auditors.

The Annual General Meeting was followed at 6.45 p.m. by the sixty-ninth Ordinary Meeting of the Group. Dr. G. W. C. Milner, F.R.I.C., A.Inst.P., was in the Chair and a lecture on "The Design of Optical Instruments for Chemical Analysis" was given by the retiring Chairman, R. A. C. Isbell, A.Inst.P.

Spectrofluorimetry of Lubricating Oils: Determination of Oil Mist in Air*

By C. A. PARKER and W. J. BARNES

(*Admiralty Materials Laboratory, Holton Heath, Poole, Dorset*)

The fluorescence emission and excitation spectra of a variety of lubricating oils have been investigated. All samples showed an intense fluorescence in the ultra-violet region, and this was made the basis of a sensitive method for the determination of oil mist in air. Less than 1 μg of oil mist per litre of air can be determined.

Low-pressure air produced by many compressors contains appreciable amounts of entrained oil mist. For certain purposes this contamination is objectionable, and to permit comparative tests to be carried out on various compressors an analytical method was required for the determination of oil mist present to the extent of less than 1 μg per litre of air at N.T.P. Preliminary tests suggested that it would be difficult to obtain the desired sensitivity by infra-red or ultra-violet absorption spectroscopy, and the possibility of a fluorimetric method was therefore investigated.

Fluorescence spectra of lubricating oils

The well known blue or green fluorescence obtained from mineral oils by excitation with "black light" of frequency 2·73 μ^{-1} (366 mμ) is comparatively weak. Much stronger fluorescence was observed in the ultra-violet region by using light of frequency greater than 3·30 μ^{-1} (303 mμ) for excitation. To determine to what degree this fluorescence was characteristic, a recording spectrofluorimeter[2] was used for investigating the fluorescence emission and excitation spectra of a variety of lubricating oils. The samples included an automobile back-axle oil, various grades of engine oil and a thin cycle oil (see Table I), as well as the lubricating oils used in the compressors to be tested.

Table I

Relative fluorescence intensities of lubricating oils

Sample No.	Description of sample	Fluorescence intensity at 2·8 μ^{-1} (357 mμ): Excited by 4·03 μ^{-1} (248 mμ)	Fluorescence intensity at 2·8 μ^{-1} (357 mμ): At excitation maximum
1	Back-axle oil	100	100
2	Commercial engine oil	81	89
3	Commercial engine oil	45	58
4	Bicycle oil	36	123
5	High-grade engine oil	21	31
6	High-grade engine oil	19	28
7	High-grade engine oil	13	22

All samples showed a fluorescence emission band with maximum in the region of 2·8 μ^{-1} (357 mμ). Typical spectra obtained by excitation with light of frequency 4·03 μ^{-1} (248 mμ) are shown in Fig. 1. The spectra are all similar, although the shape and precise position of the maximum vary from one sample to the next. Apart from the peak at 2·8 to 2·9 μ^{-1} (357 to 345 mμ), a marked inflexion at 3·2 μ^{-1} (313 mμ) can be seen in all spectra. With the exception of sample No. 4 (cycle oil) there was little fluorescence at wave numbers greater than 3·4 μ^{-1} (294 mμ). (The small sharp maximum at 3·74 μ^{-1} (267 mμ) was shown by all samples and was due to the main Raman band of the solvent.[3]) For any one sample, the shape of the fluorescence emission spectrum varied with the wavelength of excitation, although the main peak appeared roughly in the same region. For example, the emission spectra of one oil obtained by excitation with 4·03-μ^{-1} (248 mμ) and 3·5-μ^{-1} (286 mμ) light are shown in Fig. 2. With the latter wavelength, the inflexion at 3·2 μ^{-1} (313 mμ) increased

* Presented at the meeting of the Society on Wednesday, November 4th, 1959.

in intensity and a new inflexion in the region of 3·0 μ^{-1} (333 mμ) appeared. It was thus clear that the ultra-violet fluorescence bands of the oils were due (as might be expected)

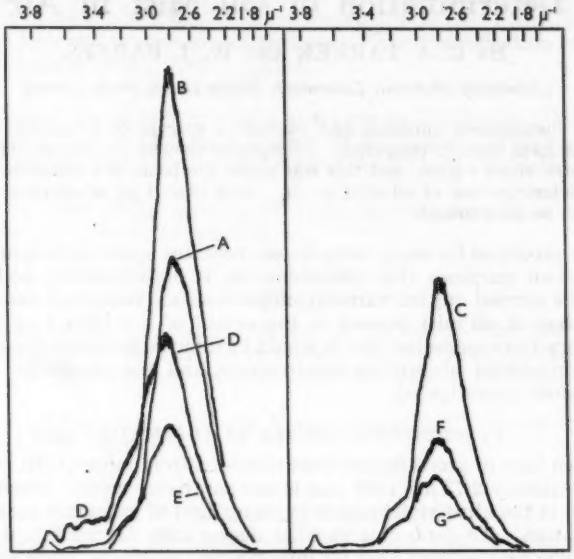

Fig. 1. Fluorescence emission spectra of 5μg per ml of lubricating oils in *cyclo*hexane with excitation at 4·03μ^{-1} (248 mμ): curve A, sample No. 1; curve B, sample No. 2; curve C, sample No. 3; curve D, sample No. 4; curve E, sample No. 5; curve F, sample No. 6; curve G, sample No. 7

(Sample Nos. refer to oils listed in Table I, p. 3; sample No. 1 measured at half sensitivity)

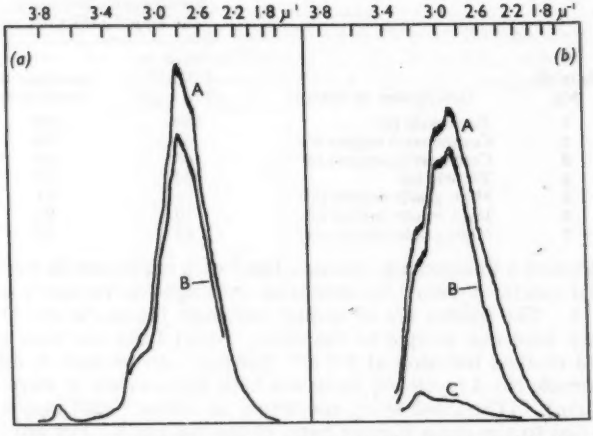

Fig. 2. Fluorescence emission spectra of compressor lubricant with excitation at (*a*) 4·03μ^{-1} (248 mμ) and (*b*) 3·5μ^{-1} (286 mμ): curves A, oil from sump of compressor; curves B, oil collected from air; curve C, filter-paper blank

to a mixture of fluorescent components in which relative proportions varied from sample to sample. This conclusion was also confirmed by comparison of the excitation spectra.

The excitation spectra (see Fig. 3) were measured by setting the fluorescence monochromator with wide slits (half-band width approximately 0·2 μ^{-1}) at 2·8 μ^{-1} (357 mμ). Qualitatively they were all similar, showing a main maximum in the region 4·2 to 4·3 μ^{-1} (238 to 233 mμ) and subsidiary maxima at 3·8 to 3·95 μ^{-1} (263 to 253 mμ) and 3·5 μ^{-1} (286 mμ), the relative intensities of the three bands varying from sample to sample. The cycle oil (sample No. 4) showed the greatest variation (see Table I). The excitation spectra were in outline similar to the absorption spectra (measured at a concentration 100 times greater), although there was little or no decrease in absorption at wave numbers greater than 4·2 μ^{-1} (238 mμ) compared with the sharp fall in this region in the excitation spectra. Samples Nos. 1, 2 and 3 showed almost identical absorption spectra, which were about twice as intense as the spectra from the high-grade oils (samples Nos. 5, 6 and 7). The cycle oil was again exceptional in showing an absorption spectrum considerably more intense than those of the remainder.

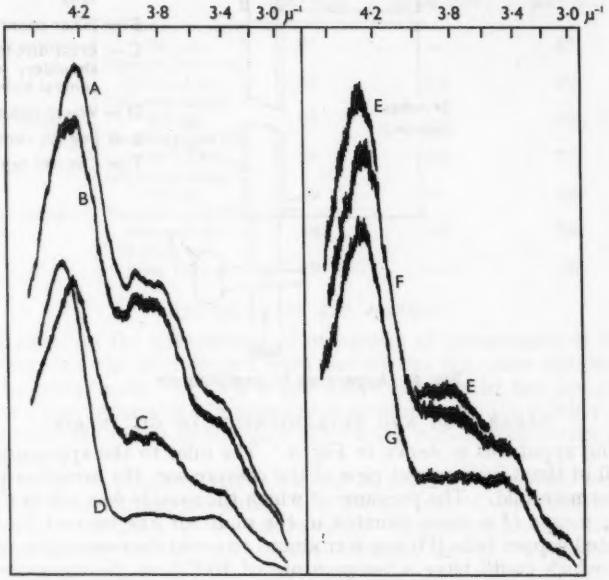

Fig. 3. Fluorescence excitation spectra of 5μg per ml of lubricating oils in *cyclo*hexane with fluorescence observed at 2·8μ^{-1} (357 mμ)
(Curves lettered as for Fig. 1; samples Nos. 1, 2 and 3 measured at unit sensitivity, sample No. 4 at half sensitivity and samples Nos. 5, 6 and 7 at unit sensitivity × 3)

The relative intensities of fluorescence of the various samples are shown in Table I. With the exception of the cycle oil, the intensities fall in the same order for both excitation wavelengths. The fluorescence of the three high-grade engine oils is less than that of the commercial engine oils, and it seems likely therefore that it is due to constituents of the refined oils themselves rather than to special additives, which are presumably present to the greater extent in the high-grade oils. In the absence of information about the methods of refining and the additives employed by the various manufacturers, it is fruitless to speculate further at this stage on the nature of the compounds causing the fluorescence, but it would obviously be of considerable interest to make measurements on mineral oils and additives whose detailed histories and methods of preparation were known.

For analytical purposes, the measurement of fluorescence emission and excitation spectra appears to provide a useful, sensitive and qualitative test for lubricating oil. However, the fluorescence intensities vary by a factor of 8, even within the small number of samples tested, and to provide anything more than a rough quantitative estimate of oil content, it is obviously necessary to have available a sample of the lubricant involved, so that the

spectrofluorimeter can be calibrated. This presented no difficulty in the work described, since a sample could be taken from the sump of the compressor immediately after sampling the compressed air.

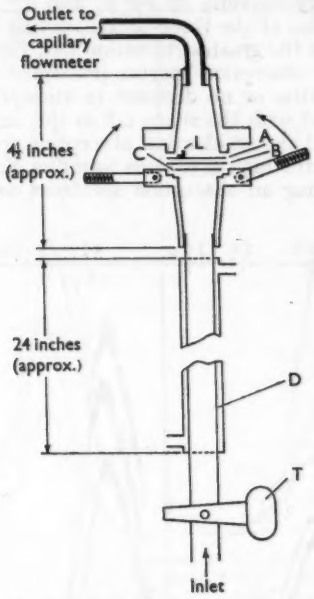

Fig. 4. Apparatus for sampling air

Separation and determination of oil in air

The sampling apparatus is shown in Fig. 4. The inlet to the apparatus was connected through the wall of the 3-inch output pipe of the compressor, the sampling point being close to the compressor manifold. The pressure at which the sample was taken (5 lb per sq. inch) was adjusted by means of a valve situated in the main air line beyond the sampling point. The water-jacketed copper tube (D) was introduced to avoid decomposition of the filter-paper by the hot air, which could have a temperature of 100° C at the compressor outlet. The filter-paper (B) was held tightly between plates (A and C) having central holes 1 cm in diameter. The flow of air was thus restricted to this area of the filter-paper. After collection from a suitable volume of air (flow rate 2·5 to 5 litres per minute) the central portion of the filter-paper was eluted with Specpure *cyclo*hexane, the solution was made up to 5 ml and its fluorescence emission and excitation spectra were measured. The spectra were then compared with those obtained by using a known concentration of the sample of oil taken from the sump of the compressor immediately after sampling the air. The filter-papers used were pre-washed with *cyclo*hexane and a blank test was carried out on an unexposed area of the paper (see Fig. 2, curve C). Tests in which the air was passed through two papers in succession showed that a negligible amount of oil was collected on the second paper.

Some typical fluorescence emission spectra obtained are shown in Fig. 2, and the results of some compressor tests are given in Table II. The results obtained on the same samples with different wavelengths for excitation were in good agreement. It is interesting to note that air from the compressor fitted with an oil-impregnated felt filter on the inlet side contained a similar amount of oil to that obtained from compressors fitted with dry paper filters.

Visual determination

Although the visible fluorescence of the oil collected on the filter-paper was not sufficiently intense to distinguish it readily from the natural fluorescence of the paper itself, it was found that, by development with ligroin in a Weiss ring oven, the oil could be concentrated in a

narrow ring and as little as 1 μg could be distinguished. By comparison with standard papers prepared by developing known weights of oil taken from the sump of the compressor, it was possible to obtain an approximate visual estimate of the oil content. This simple test had the advantage that the spectrofluorimeter was not required, but it could not of course identify the fluorescence observed as being due to oil. The results obtained by this method were in reasonable agreement with those obtained by spectrofluorimetry.

Table II

Oil contents of air from compressors

Compressor	Sample	Fluorescence with excitation at $3{\cdot}5\mu^{-1}$ (286 mμ)	Oil content of air, μg per litre	Fluorescence with excitation at $4{\cdot}03\mu^{-1}$ (248 mμ)	Oil content of air, μg per litre
No. 1, with new oil in sump—paper air filter	Oil from sump (50 μg)	47	—	47	—
	Sample from 84 litres of air	39	0·5	40	0·5
No. 2, with old oil in sump—paper air filter	Oil from sump (50 μg)	62	—	69	—
	Sample from 32 litres of air	24	0·6	31	0·7
No. 3, with new oil in sump—oiled-felt air filter	Oil from sump (50 μg)	49	—	46	—
	Sample from 42 litres of air	29	0·7	28	0·7
	Oil from air filter (50 μg)	29	—	26	—

Discussion of the method

The method relies on the fluorescence of one group of components of the oil, and it is assumed, therefore, that the oil collected from the air has the same composition as the oil in the sump of the compressor. Since it is probably only oil mist and not true vapour that is collected on the filter-paper this is a reasonable assumption. Its validity is confirmed by the results obtained in the visual tests, which rely on the visible fluorescence from a different group of components.

The proportion of oil present as true vapour is not known. With very old oils that have undergone much decomposition during use, the amount of vaporisation due to volatile decomposition products is likely to be considerable. Consideration was therefore given to the possibility of measuring the total oil content, including vapour, by passing the air through a cooled trap. This procedure was less convenient and was finally rejected on the grounds that fluorescence measurements would not provide a true measure of the vaporised decomposition products. For the purpose of comparing the performances of different compressors, the method described was adequate, and the difficulties that can be introduced by decomposition products of the oil can be avoided by using a new oil for each test. In fact, results obtained with old oil were similar to those with fresh oil.

The sensitivity of the method is limited by the filter-paper and solvent blank value, which corresponded to about 3 μg of oil (this figure could probably be decreased by more careful purification of the paper). If it is assumed that 50 litres of air are sampled (requiring 10 minutes), this blank corresponds to 0·06 μg of oil per litre of air.

We thank Mr. W. T. Rees, who provided the absorption spectra of the oils. This paper is published with the permission of the Superintendent, Admiralty Materials Laboratory.

References

1. Parker, C. A., *Nature*, 1958, **182**, 1002.
2. ——, *Analyst*, 1959, **84**, 446.

Received *July 9th*, 1959

Discussion

Mr. A. H. Gunn asked if, as the sensitivity of the method was limited by the fluorescence of the materials extracted from the filter-papers, the use of previously extracted filter-papers would improve the sensitivity.

DR. PARKER said that the blank fluorescence was due not only to small amounts of fluorescent material extracted from the filter-paper but also to traces of fluorescent impurity in the *cyclo*hexane. They did in fact pre-wash the filter-papers with *cyclo*hexane and the total fluorescent blank was then small enough for their purpose (*i.e.*, it corresponded to less than 0·1 μg of oil per litre of air). It could no doubt be reduced still further by more exhaustive extraction of the filter-paper and by purification of the *cyclo*hexane. It had incidentally been noticed that many samples of *cyclo*hexane, specially purified for absorption spectroscopy and showing low absorption throughout the ultra-violet region, nevertheless showed quite intense and complicated fluorescence spectra.

MR. J. F. HINSLEY referred to the diagram of the spectrofluorimeter, which showed a fluorescent device used for monitoring the output of the source of exciting radiation. He asked Dr. Parker to give further details of this device and to say whether its efficiency was independent of the wavelength of the radiation emitted by the source.

DR. PARKER replied that the fluorescent-screen monitoring device used in the spectrofluorimeter had been described in a paper already published (reference 1, above). It consisted of an alkaline solution of fluorescein (4×10^{-3} *M*) contained in an optical cell of depth 5 mm, the fluorescence excited by the incident light being viewed through the back face of the cell. The quantum efficiency of the device was checked against the ferrioxalate actinometer and its quantum yield was found to be constant to within ± 10 per cent. over the wavelength range 230 to 500 mμ.

MR. A. G. HILL asked whether the authors were satisfied that the filter-paper retained all the oil mist.

DR. PARKER stated that, in experiments in which two filter-papers were used in series, a negligible amount of oil was collected by the second. It was therefore assumed that all the oil mist had been trapped by the first filter-paper. It was not known, however, what proportion of oil present in the form of true vapour was collected by the filter-paper.

The Assay of Acetylcholine with the Isolated Semispinalis Cervicis Muscle of the Chick

BY CHRISTINE TYLER*

(*Department of Pharmacology, Royal Free Hospital School of Medicine, 8 Hunter Street, London, W.C.1*)

A method is described in which the semispinalis cervicis muscles of 2- to 3-week-old chicks are used for the rapid and accurate assay of acetylcholine. The preparation is sensitised to acetylcholine by adding edrophonium chloride to the bath to give a concentration of 4 to 8 μg per ml before each injection of acetylcholine. Ten assays of a (2,2) design were carried out on solutions of known potency. The mean error was $\pm 2{\cdot}8$ per cent., the mean fiducial limits of error were 7·3 per cent., and the mean index of precision was 0·031.

IT was shown by K. Child and E. Zaimis, in unpublished work, that the semispinalis cervicis muscle of the chick, like the rectus abdominis muscle of the frog, responds by contracture to acetylcholine and substances possessing an acetylcholine-like action at the neuromuscular junction. For assay purposes, the chick muscle has one main advantage over the rectus abdominis of the frog; the relaxation following the contracture is much more rapid, so that the time interval between doses is shorter and the total experimental time is reduced.

EXPERIMENTAL

ANIMALS—

Light Sussex crossed Rhode Island Red pullets, 14 to 21 days old, were used. They were received on the day of hatching and were housed in a container maintained at 85° to 90° F.

PREPARATION—

For the dissection of the semispinalis cervicis muscle, the method described by Child and Zaimis was used. The chick is anaesthetised with ether and plucked on the dorsal side of the neck. A longitudinal incision is made in the skin from the base of the skull to the second thoracic vertebra. The neck is slightly arched by means of a small pad of cotton-wool,

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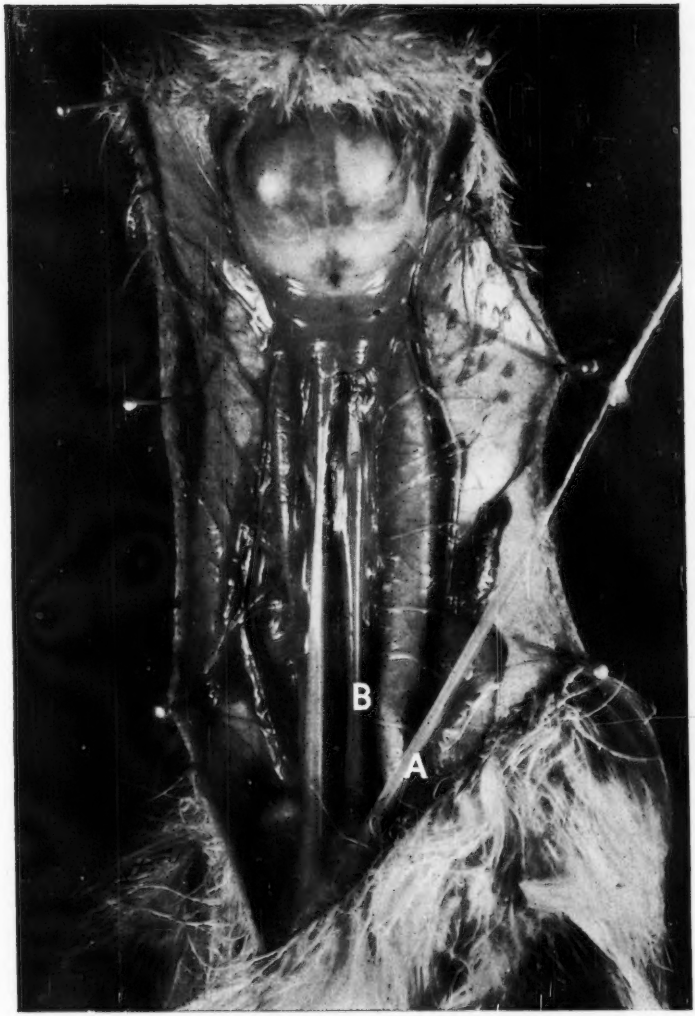

Fig. 1. Dissection of the dorsal aspect of the neck of a chick, showing the right biventer muscle (A) retracted to expose the right semispinalis cervicis muscle (B) (reproduced from Child[1])

[To face p. 8.

and the connective tissue is removed to expose the two biventer cervicis muscles. The biventer cervicis muscle of one side is separated from the semispinalis cervicis muscle, which is directly beneath it (see Fig. 1). The semispinalis cervicis muscle is then dissected away from the oblique muscles of the neck, and a thread is tied round each end of the muscle before it is removed from the body. The isolated muscle is then suspended as rapidly as possible in a 5-ml isolated-organ bath containing oxygenated Tyrode solution at 40·5° C.

Child and Zaimis developed this preparation for assaying acetylcholine-like substances acting at the neuromuscular junction. In this paper a further development is described for the assay of acetylcholine itself. Chicks from 3 to 7 days old were used by Child and Zaimis for assaying decamethonium, but preparations from chicks of this age were unpredictable in their responses to acetylcholine. When the chicks were from 14 to 21 days old, however, the responses to acetylcholine were more regular; consequently, chicks from 14 to 21 days old were used throughout the experiments described here.

Choice of anticholinesterase drug—

The preparation is relatively insensitive to acetylcholine unless it has been previously treated with an anticholinesterase drug,[1] possibly because of the high concentration of cholinesterase within the muscle (unpublished work by F. Hobbiger, 1953). The anticholinesterase drug was tested either by inclusion in the Tyrode solution, so that the muscle was continuously under its influence, or by adding it to the contents of the isolated-organ bath at a fixed interval before the acetylcholine was added. Several anticholinesterase drugs were tested, *i.e.*, physostigmine, neostigmine, di*iso*propylfluorophosphonate, tetraethylpyrophosphate and edrophonium chloride. Each of these drugs potentiated the effect produced by acetylcholine, but the first four were unsuitable, as they either produced too small an increase in sensitivity or caused a partial contracture of the muscle, from which it did not relax completely.

Edrophonium chloride, however, was satisfactory for assay purposes. It was not included in the Tyrode solution, since, when used in this way, it caused long-lasting contracture even at extremely low concentrations; however, when injected into the bath to produce a concentration of 4 to 8 μg per ml, before each addition of acetylcholine, it caused a satisfactory increase in sensitivity and no contracture. When the preparation was so treated, recovery after doses of acetylcholine was rapid and complete, and there was no evidence of tachyphylaxis. All preparations were sensitive to about 0·2 μg of acetylcholine and some responded to 0·02 μg.

Assay procedure—

Each dose of acetylcholine was left in contact with the muscle for 75 seconds, and edrophonium chloride was added 30 seconds before each injection of acetylcholine. The interval between doses of acetylcholine was 4 minutes. The assays were of a (2,2) design and the four doses in each group were given in random order.[2] The ratio of the large dose to the small dose was either 3 to 2 or 4 to 3.

Ten assays were carried out in this way on solutions having known potencies, so that the accuracy of the method could be determined. Each assay was performed on the muscle from a different chick.

Results

The tracing obtained in a typical assay (assay No. 5, see Table III) is shown in Fig. 2; the measured contractures are shown in Table I, and the results are presented graphically

Table I

Heights of contractures in the four groups of assay No. 5

Solution	Dose	Height of contracture in— group 1, mm	group 2, mm	group 3, mm	group 4, mm	Sum of heights, mm
Test	Large (0·3 ml)	27	25	24	20	96
Standard ..	Large (3·0 μg)	29	27	27	30	113
Test	Small (0·2 ml)	12	14	10	12	48
Standard ..	Small (2·0 μg)	16	19	15	14	64
	Sum	84	85	76	76	321

in Fig. 3. The responses are fairly constant, the regression is good, and there is little deviation from parallelism. An analysis of variance of this assay is shown in Table II, from which it can be seen that neither the variation between groups nor the deviation from parallelism

TABLE II

ANALYSIS OF VARIANCE OF ASSAY No. 5

Source of variation	Degrees of freedom	Sum of squares	Variance	Value of F	Value of P
Groups	3	17·00	5·67	1·35	>0·05
Standard and unknown	1	68·10	68·10	16·20	<0·01
Regression	1	588·00	588·00	139·80	<0·001
Deviation from parallelism ..	1	0·06	0·06	0·01	>0·05
Residual error	9	37·84	4·20	—	—
Total	15	711·00			

$S_m = 0{\cdot}0157$ $b = 54{\cdot}7$ $s = 2{\cdot}05$ $s/b = 0{\cdot}037$

Fiducial limits = 8·2 per cent.

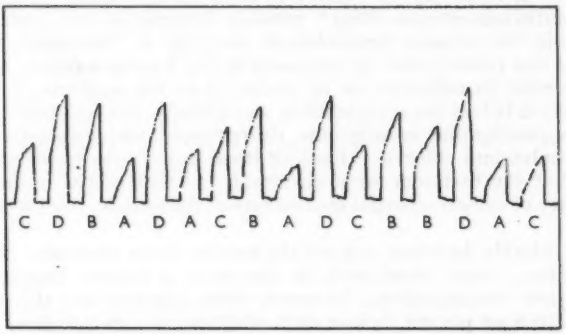

Fig. 2. Tracing from a typical assay of acetylcholine with the semispinalis cervicis preparation: A, 0·2 ml of test solution; B, 0·3 ml of test solution; C, 2·0 μg of acetylcholine; D, 3·0 μg of acetylcholine

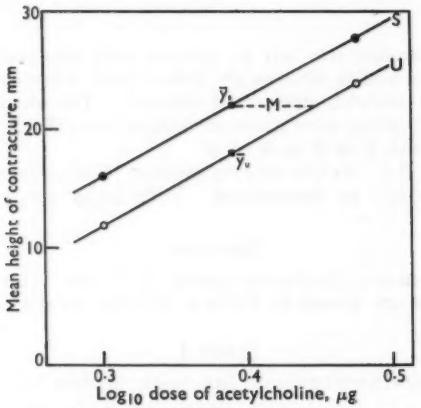

Fig. 3. Graphical representation of results of assay shown in Fig. 2

is significant (P>0·05), whereas the regression between the large and small doses is highly significant (P<0·001).

The results of the ten assays are summarised in Table III; the average difference between the known potency and that found is ±2·8 per cent., the average fiducial limits are ±7·3 per cent., and the average index of precision (s/b) is 0·031.

TABLE III

RESULTS OF TEN ASSAYS ON SOLUTIONS OF KNOWN POTENCY

Assay No.	Ratio of large dose to small dose	Potency, μg per ml	Potency found, μg per ml	Difference, %	Fiducial limits, %	Value of s/b
1	3 to 2	7·0	6·6	4·8	12·3	0·054
2	4 to 3	10·0	9·8	1·7	6·0	0·018
3	3 to 2	10·0	9·7	3·0	5·3	0·030
4	4 to 3	9·1	9·0	0·8	13·3	0·038
5	3 to 2	8·7	8·7	0·6	8·2	0·037
6	3 to 2	9·3	9·1	2·6	4·1	0·023
7	4 to 3	8·5	8·8	4·1	7·0	0·021
8	3 to 2	8·5	9·0	5·9	5·9	0·022
9	3 to 2	11·0	10·8	1·8	3·6	0·032
10	3 to 2	11·2	11·5	2·6	7·4	0·029
		Mean		2·8	7·3	0·031

DISCUSSION OF RESULTS

Among the most popular biological methods for assaying acetylcholine are those in which the rectus abdominis muscle of the frog,[3,4] the longitudinal muscle of the leech[4,5] or the blood pressure of the cat[6] is used. The characteristics of these three methods and of the proposed method are summarised in Table IV. The longitudinal muscle of the leech provides a valuable preparation for identifying acetylcholine and for approximate quantitative work when high sensitivity is required, but the method is intolerably slow when many samples must be tested. The method involving use of the blood pressure of the cat is the most sensitive, and an assay can be rapidly completed. The accuracy is satisfactory, but the method has the disadvantage that not all cats are suitable.

TABLE IV

CHARACTERISTICS OF METHODS AVAILABLE FOR ASSAYING ACETYLCHOLINE

The results for the first three methods were published by MacIntosh and Perry[7]

Method	Speed of assay	Accuracy*	Threshold dose of acetylcholine, μg
Longitudinal muscle of leech ..	Slow	To within ±15 to 20 per cent.	0·005
Rectus abdominis muscle of frog	Adequate	To within ±5 per cent.	0·05
Blood pressure of cat	Rapid	To within ±10 per cent.	0·003 to 0·006
Semispinalis cervicis muscle of chick	More rapid	To within ±5 per cent.	0·02

* These figures indicate standard deviations for successive comparisons (two doses of acetylcholine and two doses of extract per comparison).

The frog rectus abdominis preparation is probably the most convenient and accurate of the methods hitherto available for work in which high sensitivity is not required. It seems, however, that the use of the proposed preparation has definite advantages over it. First, the sensitivity of the chick muscle is slightly greater and stays constant for several hours, whereas in the frog muscle preparation there is often a slow steady decrease in sensitivity. Secondly, biological fluids, such as blood, plasma or urine, have little effect on the tone of the chick muscle preparations, and acetylcholine in such fluids need not be extracted before the assay. Thirdly, the speed with which an assay can be carried out with the chick muscle preparation is much greater than that with the frog muscle preparation. This is because the chick muscle relaxes rapidly; artificial stretching is never required.

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Micro Method for the Determination of Calcium and Magnesium in General Biological Material

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A micro method for the determination of calcium and magnesium in general biological material is described and critically examined.

A simple quantitative device for the comminution of tissues is described, with a method of treating the ground material with trichloroacetic acid. An alternative ashing procedure is also described.

The calcium and magnesium are precipitated together as phosphates from the trichloroacetic acid filtrate or from the solution of the ash. Calcium and magnesium are shown to be thus quantitatively recoverable at or above concentrations of 7×10^{-6} and $2{\cdot}4 \times 10^{-6}$ mole per litre, respectively. The metals are thus separated from excess of phosphate and iron and from copper and zinc. The solution of the precipitate is often directly titratable for calcium and calcium plus magnesium by the method already described by Hunter.

Interfering metals, which may under certain conditions accompany the phosphate precipitate, are removed from its solution by treatment with diethyldithiocarbamic acid and extraction with carbon tetrachloride. Diethyldithiocarbamate does not compete with the metal indicators for calcium and magnesium, and, as the solubility of carbon tetrachloride in water is negligible, there is no volume change in the aqueous phase.

In order to test the method, analyses by different procedures of biological materials with widely varying chemical compositions are given. The particular procedure chosen depends on the nature of the material to be analysed and for most materials may be made a simple routine operation.

A MICRO method has already been described for the determination of magnesium in presence of calcium with the metal indicators murexide and Eriochrome black T.[1] The method is applicable to simple mixtures of calcium and magnesium and to biological fluids such as blood plasma and cerebrospinal fluid after de-proteinisation.[2] It is not applicable to urine or to muscle, nerve and most animal organs, owing especially to interference from the presence of excessive amounts of phosphate. With ashed tissues of animal origin the extra problem of removing the iron from haemoglobin arises, and other possible interfering metals, such as copper, zinc and manganese, may be present in the ash of tissues from either plant or animal. It would thus appear that any method suitable for determining calcium and magnesium in biological material with the metal indicators currently in use must include satisfactory means for the separation of these alkali metals from phosphate ions and from other likely interfering metals. It is further required that, in the treatment of tissues for analysis, no added reagent that competes with the metal indicators in complexing calcium or magnesium may be present in the final test solutions, and reagents must not contain significant amounts of these ubiquitous elements.

Several methods have been suggested for the removal of phosphate. Collier[3] added a large excess of molybdic acid and extracted with chloroform. A similar principle has been suggested for urine by Horner,[4] who used a large excess of morpholine and precipitated with tungstic acid both morpholine phosphotungstate and tungstate. To remove excess of phosphate from plant ash Padhye[5] has used zirconium nitrate, and Ling[6] for milk and whey has used metastannate. With animal-tissue ash Hamm[7] and Griswold and Pace[8] used ion-exchange resins. Several of these methods have been tried, but none was found suitable for the determination of calcium and magnesium in micro amounts.

The removal of iron with phosphate or with acetate and heat is not sufficiently complete to prevent interference with the Eriochrome black T indicator at pH 10·2.

Our method for the determination of magnesium in blood serum,[2] in which the metal is not precipitated, yielded values in the condition of bovine hypomagnesaemia no higher than those obtained by Denis's method as used in the Biochemistry Department, Central

Veterinary Laboratory, Weybridge. This indicated that the ammonium magnesium phosphate must be exceedingly insoluble, and I thank Mr. Salt of that Department for supplying details of the conditions of precipitation used there.

This observation has led to a study of the precipitability as phosphates of both calcium and magnesium together. Evidence will be given that as little as 1 μg of calcium and 0·2 μg of magnesium are quantitatively precipitable from a volume of 3·5 ml in presence of excess of phosphate and ammonia. Such a degree of insolubility appeared adequate, and, as appreciably more than the molecular equivalent of phosphate combined with the metals does not significantly interfere with either the murexide or Eriochrome black T indicators, the method of determining magnesium in presence of calcium, already described, could be applied to the solution of the precipitate. Excess of phosphate in the original material and in the reagents would thus be discarded in the supernatant fluids and washings of the phosphate precipitate.

This device serves also to remove excess of iron. In presence of excess of phosphate, iron is not precipitable as hydroxide in presence of excess of ammonia, and the solubility of ferric phosphate is such that even with ashed erythrocytes only traces of iron are precipitated with the calcium and magnesium phosphates, and such traces can be removed by treatment with diethyldithiocarbamic acid and carbon tetrachloride without loss of the alkali metals (see p. 18). It may further be noted that any copper or zinc present will likewise remain in the supernatant fluid from the phosphate precipitation. The advantages of precipitating calcium and magnesium as phosphates are thus obvious.

Phosphate precipitation is carried out either on solutions of ashed samples or on trichloroacetic acid extracts of tissues. The phosphate precipitate readily dissolves in dilute acid, and often this solution may be titrated directly for calcium and for calcium plus magnesium. When interfering metals are present the solution is treated with diethyldithiocarbamic acid and extracted with carbon tetrachloride.

The values in Table V (see p. 19) are given as evidence that the method is a comprehensive one, rather than as a measure of the precision attainable with materials differing greatly in chemical composition; we have, however, had little experience of testing many of the materials listed in Table V. It will be seen that the method of choice is simple and readily applicable to routine use in a variety of fields.

Method

Apparatus—

The colour comparator* used has already been briefly described.[1]

Hot-plates—Two hot-plates with Simmerstat controls are used. One is maintained at 80° to 150° C for preliminary evaporation and oxidation of materials to be ashed and the other at about 300° C for oxidation according to Middleton and Stuckey's principle.[9]

Tissue grinder and extractor—Most plant and animal tissues, in amounts requisite for analysis, are readily comminuted with the glass mortar and pestle shown in Fig. 1. The rounded pestle is ground with carborundum to engage with the total bottom surface of the mortar. The Pyrex-glass tube is 16 mm × 115 mm.

Micro ashing flasks—These have already been described.[10] They are tared for convenience in adjusting solutions of ash to required volumes.

Centrifuge tubes—E-Mil plastic-stoppered graduated or plain 10-ml tubes.

Pipettes—E-Mil automatic micropipettes, 0·02 to 0·25 ml, and other graduated pipettes.

Reagents—

It may be assumed that all commercial reagents contain at least traces of calcium and magnesium; in work on a micro scale, these may be a source of gross error. It is therefore necessary to have the three main reagents, water, nitric acid and trichloroacetic acid, as free as possible from metals. They were prepared and tested as described below.

Water—Water from a Manesty still run through an Elgastat de-ioniser has proved reliable. It was tested by adding 1 or 2 ml to one cup of the zeroed murexide and Eriochrome black T indicator solutions in the colour comparator.

* The colour comparator is the subject of patent applications, the rights in which are the property of the National Research Development Corporation, 1 Tilney Street, London, W.1, to whom enquiries about the source of supply of the apparatus should be directed.

Nitric acid—AnalaR nitric acid, sp.gr. 1·42, was gently distilled from a flask attached to an upright empty reflux condenser about 20 inches long to prevent liquid carry-over into the attached horizontally inclined water-cooled condenser. The distillate was tested as described under "Procedure," and it was found that 1 ml contained 0 to 0·1 μg of calcium and 0·40 to 0·50 μg of magnesium (or their equivalents of interfering metals). This gives negligible or extremely small blank values for both calcium and magnesium with the test portions used for most materials.

Trichloroacetic acid—AnalaR trichloroacetic acid was distilled under reduced pressure in the apparatus described for nitric acid. A 10 per cent. w/v solution was prepared and tested as described under "Procedure," and it was found that 1 ml contained 0·5 μg of calcium and 0·15 μg of magnesium (or their equivalents of interfering metals).

Fig. 1. Tissue grinder and extractor (one-third full size)

Ammonium hydroxide—AnalaR ammonia solution, sp.gr. 0·88, was found to contain 0·5 μg of calcium and 0·15 μg of magnesium per ml (or their equivalents of interfering metals) and was regarded as satisfactory.

Ethanol—One millilitre of absolute ethanol (Burrough's) was found to contain about 0·5 μg of calcium and less than 0·1 μg of magnesium and was regarded as satisfactory.

Ethanolic ammonia solution—One millilitre of ammonia solution, sp.gr. 0·88, was added to 100 ml of 70 per cent. v/v ethanol.

Ammonium phosphate solution—One-hundred millilitres of 0·2 *M* diammonium hydrogen orthophosphate (AnalaR) were mixed with 150 ml of water and 100 ml of ammonia solution, sp.gr. 0·88. The mixture was set aside at about 4° C for several days, and the supernatant fluid was then removed by decantation; it was nearly free from calcium and magnesium.

Sodium diethyldithiocarbamate solution—A 0·1 *M* solution of the AnalaR reagent was spun in a centrifuge to remove a small insoluble residue.

Carbon tetrachloride—About 500 ml of AnalaR carbon tetrachloride were shaken with 20 ml of 0·1 *N* nitric acid. The aqueous layer was removed, and the organic layer was washed twice with water.

PROCEDURE—

Reagent-blank values—Place measured amounts of nitric acid, trichloroacetic acid and ammonia solution in micro ashing flasks, and evaporate to dryness on the cooler hot-plate. Ash, if necessary, with a minimum amount of nitric acid, as described below; then dissolve the ash in dilute nitric acid, dilute to a definite volume, and titrate with murexide and Eriochrome black T.

Test other reagents, such as sodium diethyldithiocarbamate, similarly.

Materials in general—The procedure to be adopted for the determination of calcium and magnesium will depend on the nature of the sample. With most materials the analyst has the alternatives of ashing the material or extracting it with trichloroacetic acid. Treatment with trichloroacetic acid is preferable to ashing for several reasons. Trichloroacetic acid filtrates of animal tissue, and particularly of whole blood or erythrocytes, contain none of the iron from haemoglobin. In our experience the filtrates contain all the calcium and magnesium found to be present in the corresponding ashed materials. Our limited observations on plant material containing chlorophyll also suggest that all the magnesium present is released to the trichloroacetic acid extract. Trichloroacetic acid filtrates are, however, unsuitable for titration with the metal indicators because of their high acidity and, if this is overcome merely by neutralisation, because of the serious complexing effect of the trichloroacetate present. The trichloroacetic acid filtrates may be treated in either of two ways; by ashing or, preferably, by precipitation with ammonium phosphate.

Arguments for ashing are: (*a*) the certainty that all organic material is destroyed; if free from excess of phosphate and interfering metals the solution of the ash may be titrated directly, (*b*) it dispenses with the need for grinding, extracting and measuring aliquots of

spun supernatant fluid and (*c*) it avoids the use of trichloroacetic acid, which has the disadvantages noted above, and likely additions to the blank value.

Both methods of treatment can be illustrated with human washed packed erythrocytes, which contain excesses of phosphate and iron and may be regarded as "difficult" material.

Ashing—Place two 0·25-ml portions of erythrocytes in separate micro ashing flasks, and oxidise with concentrated nitric acid, as described for blood serum.[11] Carry out at the same time duplicate blank tests containing like amounts of all reagents. The process is rather more protracted than with serum and requires about 0·5 ml of acid to complete the oxidation. To the ash add 1 ml of 1 *N* nitric acid, and heat under reflux for at least 1 hour on a hot-plate at 80° to 90° C, but avoid heating to dryness by adding water if necessary. Transfer the solution to a centrifuge tube, and wash the flask with 1 and then 0·5 ml of water. To the solution of the ash, about 3·0 ml, add 1·5 ml of diammonium hydrogen orthophosphate solution and 0·75 ml of ammonia solution, sp.gr. 0·88. Mix, insert the stopper, and set aside at about 4° C for at least 24 hours.

Spin in a centrifuge, carefully decant the brownish supernatant fluid, and rest the inverted tube over filter-paper for a short time. Then place in the tube 1·5 ml of ethanolic ammonia solution, mix, and again spin in a centrifuge. Decant the supernatant fluid, drain the tube, and add 0·25 ml of 1 *N* nitric acid. Place the tube in warm water, and add 2·1 ml of water. Mix, cool, and add 0·1 ml of sodium diethyldithiocarbamate solution. Mix, set aside for a few minutes, and then add 1 ml of carbon tetrachloride. Insert the stopper, shake, and again spin in a centrifuge. Insert the end of a pipette drawn to a capillary through the aqueous layer, and withdraw the carbon tetrachloride solution by suction with a pro-pipette. Titrate the resulting aqueous solution, representing a 1 in 10 dilution of red blood corpuscles, for calcium and magnesium with the metal indicators murexide and Eriochrome black T as already described.[1,2]

Trichloroacetic acid extraction—To 0·25 ml, in duplicate, of erythrocytes add 2·75-ml portions of water and, after complete haemolysis, 2·0-ml portions of 10 per cent. trichloroacetic acid. Mix thoroughly, and evacuate gas from the precipitate. Spin in a centrifuge, and transfer 4·0-ml portions of the supernatant fluids to centrifuge tubes. Add 2·0 ml of ammonium hydrogen orthophosphate solution and 1 ml of ammonia solution, sp.gr. 0·88. Mix, insert the stopper, and set aside at about 4° C for 24 hours. Carry out duplicate blank tests with the same reagents at the same time as tests on the blood filtrates. Spin the tubes in a centrifuge, and continue as described above under "Ashing," adding 0·15 ml of nitric acid and adjusting to a volume of 2·0 ml, without the addition of thiocarbamate.

General remarks—Either procedure can be applied to most biological materials. Some hard skeletal materials, such as bone, shells and wood, are most conveniently ashed, as also are some foodstuffs containing much fibrous tissue, carbohydrate or fat. Most plant and animal tissues, in the small amounts required for analysis, are readily comminuted in the grinding tube shown in Fig. 1. The dry tube is weighed, and a suitable amount of tissue (containing not less than 10 μg of calcium and 2 μg of magnesium, and preferably 2 to 3 times these amounts) is placed in it. If it is decided that the total volume should be 5 ml, then add 2 ml of trichloroacetic acid solution, and grind the tissue. Adjust the volume with water, assuming that the weight of tissue in grams equals the volume in millilitres. Mix with the pestle, then remove it, spin the tube in a centrifuge, and transfer a measured amount of the supernatant fluid to a centrifuge tube. Add a half volume of diammonium hydrogen orthophosphate solution and a quarter volume of ammonia solution, sp.gr. 0·88, and proceed as already described.

With many materials, especially when trichloroacetic acid filtrates are used for precipitation of the phosphates, *e.g.*, blood serum, most urines, milk and many tissues, the proportion of interfering metals present in relation to the magnesium present is so small that thiocarbamate treatment is unnecessary. Whether or not it is necessary is easily decided by taking, *e.g.*, 2 ml of the slightly acid final solution, which has already been titrated for calcium plus magnesium, adding 0·1 ml of the thiocarbamate solution and 0·2 ml of 0·1 *N* nitric acid, and extracting with 1 ml of carbon tetrachloride. One extraction is usually sufficient. Again titrate the aqueous phase, and, if the titre multiplied by 2·3/2·0 is less than the titre before treatment, then some interfering metal was originally present.

When metal interference is not significant and there is no excess of phosphate present,

as with blood serum and some other body fluids, it is sufficient to prepare de-proteinised extracts,[2] which are directly suitable for titration of both calcium and magnesium.

Tap-water may be directly titrated, or the calcium and magnesium may be precipitated as phosphate and the solution of the precipitate treated with thiocarbamate if other metals interfere.

Results

Table I shows recoveries of calcium and magnesium from the mixed phosphate precipitate from simple solution of the two elements. From a standard solution of calcium and magnesium, measured volumes were placed in duplicate in centrifuge tubes and like volumes,

Table I

THE PRECIPITATION OF CALCIUM AND MAGNESIUM, AS PHOSPHATES, FROM A VOLUME OF 3·5 ml

Calcium			Magnesium		
Present, μg	Found, μg	Difference from mean, μg	Present, μg	Found, μg	Difference from mean, μg
24·5	25·0, 24·0	0	4·5	4·6, 4·6	0·1
19·6	19·5, 19·0	0·4	3·6	3·7, 3·5	0
9·8	10·0, 9·5	0·1	1·8	1·7, 1·9	0
4·9	5·5, 5·0	0·3	0·9	1·1, 0·9	0·1
2·0	2·5, 2·0	0·2	0·4	0·5, 0·4	0·05
1·0	1·0, 1·2	0·1	0·2	0·24, 0·22	0·03

the same pipette being used, were placed in carefully washed test-tubes. The volumes of the solutions in the centrifuge tubes were adjusted to 2 ml, and 1 ml of diammonium hydrogen orthophosphate solution and 0·5 ml of ammonia solution, sp.gr. 0·88, were added. Corresponding reagent-blank solutions were placed in similar centrifuge tubes. The solutions were mixed, stoppered, and set aside at about 4° C over two nights. The tubes were then spun in an M.S.E. clinical centrifuge for about 5 minutes. The supernatant fluids were poured off, the inverted tubes drained briefly, and the contents then washed with 1·5 ml of ethanolic ammonia solution. To each of the drained tubes were added 0·15 ml of *N* nitric acid and 2·3 ml of water. The volumes of the control solutions in the test-tubes were likewise adjusted to 2·5 ml. Titrations were carried out as already described.

The above-mentioned observations have been repeated in the presence of trichloroacetic acid with similar findings. The complexing action of trichloroacetic acid at the concentration achieved by our procedure is not sufficient to interfere with the quantitative precipitation of calcium and magnesium by phosphate.

Evidence has already been given[2] that diluted plasma, adjusted to pH about 5·5 with acetic acid and heated, yielded filtrates suitable for the direct titration of calcium and magnesium with the metal indicators. Table II shows that the phosphate precipitates from

Table II

DETERMINATION OF CALCIUM AND MAGNESIUM IN PLASMA BY DIFFERENT METHODS

The figures in brackets are means

Heat and acetic acid filtrate				Phosphate precipitate from trichloroacetic acid filtrate	
Direct		Phosphate precipitate			
Calcium, mg per 100 ml	Magnesium, mg per 100 ml	Calcium, mg per 100 ml	Magnesium, mg per 100 ml	Calcium, mg per 100 ml	Magnesium, mg per 100 ml
9·8, 9·6 (9·7)	2·10, 2·10 (2·10)	9·8, 9·4 (9·6)	2·05, 2·10 (2·08)	9·4, 9·8 (9·6)	2·05

such a filtrate, and also the phosphate precipitate from a trichloroacetic acid filtrate of the same plasma, yielded values for calcium and magnesium in good agreement with those obtained by direct titration of the acetic acid filtrate.

Table III compares the values obtained in duplicate from the washed erythrocytes from three bloods by the proposed ashing and trichloroacetic acid treatments. It is evident that the relatively large amounts of iron present in the ash of erythrocytes are removed by the

TABLE III

DETERMINATIONS OF CALCIUM AND MAGNESIUM IN WASHED PACKED HUMAN ERYTHROCYTES

Determinations carried out by titration of solutions of the phosphate precipitates from solutions of ashed erythrocytes and of solutions of the phosphate precipitates from trichloroacetic acid filtrates of the haemolysed erythrocytes. The figures in brackets are means

	Phosphate precipitate of solution of ashed ethyrocytes		Phosphate precipitate from trichloroacetic acid filtrate	
Blood No.	Calcium, mg per 100 ml	Magnesium, mg per 100 ml	Calcium, mg per 100 ml	Magnesium, mg per 100 ml
625	0·2, 0·4 (0·3)	3·9, 3·9 (3·9)	0·2, 0·4 (0·3)	4·1, 3·7 (3·9)
626	0·6, 0·6 (0·6)	5·8, 5·3 (5·6)	0·8 (0·8)	5·6, 5·6 (5·6)
628	0·6, 0·4 (0·5)	5·1, 5·0 (5·1)	0·4, 0·4 (0·4)	5·0, 5·5 (5·3)
628 (10 mg of calcium added per 100 ml)			9·8, 11·0 (10·4)	

procedures described without loss of either calcium or magnesium. Table III also shows that calcium added to haemolysed erythrocytes is recoverable in the phosphate precipitate from the trichloroacetic acid filtrate.

The values in Table IV reflect the interfering effects of both metals and excess of phosphate in ashed liver. Before treating the solution of the ash with thiocarbamate the titrations with both murexide and Eriochrome black T gave higher results than the corresponding titrations after thiocarbamate treatment. The carbon tetrachloride extracts were yellow-brown in colour and no doubt contained both copper and iron, which accounts for the high values on the untreated solutions. The corresponding values obtained on the solutions of the phosphate precipitates are greater for both calcium and magnesium than the values found in the carbamate-treated solutions of ash. Such findings are accounted for by the presence of excess of phosphate in the thiocarbamate-treated solutions of the ash. Further, it can be seen from Table IV that there is no significant difference between magnesium values before and after thiocarbamate treatment of the solution of the phosphate precipitate, and the small increase in calcium values shown probably arises from errors in the extremely small titres. It is also apparent that in the phosphate precipitation of solutions of liver ash a negligible amount, if any, of interfering metal is precipitated.

TABLE IV

TITRATIONS, CALCULATED AS CALCIUM AND MAGNESIUM, BEFORE AND AFTER THIOCARBAMATE TREATMENT OF SOLUTIONS OF ASHED LIVER AND OF SOLUTIONS OF PHOSPHATE PRECIPITATES FROM THE SAME SOLUTIONS

The ash was digested for 0·5, 1·0, 1·5 and 2 hours in 1 ml of 5 *N* nitric acid in the order listed

	Calcium				Magnesium			
	Solution of ash		Solution of phosphate precipitate		Solution of ash		Solution of phosphate precipitate	
Weight, as fresh liver, mg	Before, mg per 100 mg	After, mg per 100 mg	Before, mg per 100 mg	After, mg per 100 mg	Before, mg per 100 mg	After, mg per 100 mg	Before, mg per 100 mg	After, mg per 100 mg
218	9·2	4·1	5·3	5·7	30·3	17·9	19·3	19·3
224	8·9	4·9	5·4	5·8	29·0	17·9	19·2	19·2
227	8·8	4·8	5·3	6·1	29·5	18·5	19·8	19·8
243	9·1	4·5	5·4	5·7	28·8	17·7	19·4	19·0

Table V compares the calcium and magnesium values obtained from the phosphate precipitates prepared from the solutions of a variety of ashed materials with those prepared from trichloroacetic acid extracts of the same materials. Each value given represents a

determination and is usually the mean of two or more titrations. Direct-titration values, after thiocarbamate treatment, when necessary, are also listed for some materials. A few values obtained from ashed trichloroacetic acid filtrates are also shown in Table V.

Discussion of the method

Interfering factors—

The preparation of solutions containing the total calcium and magnesium in general biological material and which are suitable for use with the metal indicators murexide and Eriochrome black T was the main objective of the work described. Suitable test solutions must be colourless, free from suspended matter, nearly neutral or only slightly acid and free from other metals that titrate with the indicators; they must have no excess of phosphate that competes with the indicators and no excess of complexing organic substances, such as citric acid; they must be free from complexing agents, such as ethylenediaminetetra-acetic acid, which quantitatively appropriates the metals from the indicators and which may be introduced into body fluids in the course of medical treatment of certain disorders.

For the quantitative removal of interfering metals the solution should be just acid to litmus paper after the addition of sodium diethyldithiocarbamate. (It may be noted that a 0·1 *M* solution of this salt is 0·2 *N*.) The metal thiocarbamates are then readily extractable with carbon tetrachloride. If solutions are highly acid, some interfering metals, such as iron, return to the aqueous phase, and calcium and magnesium also seem to be lost. Gage[11] has observed the importance of acidity in the distribution of lead and bismuth thiocarbamates between aqueous and organic phases. No unnecessary excess of the thiocarbamate is used, although the presence of relatively large amounts, *e.g.*, 0·25 ml of 0·1 *M* solution, has no competing action in either the calcium or magnesium titration. This property of the reagent is most valuable for our purposes, as a certain excess must be used, and this excess is not extractable with carbon tetrachloride. It is also most fortunate that carbon tetrachloride has a negligible solubility in water, so that when water-saturated carbon tetrachloride is used to extract the metal thiocarbamates, there is no significant change in volume of the aqueous phase.

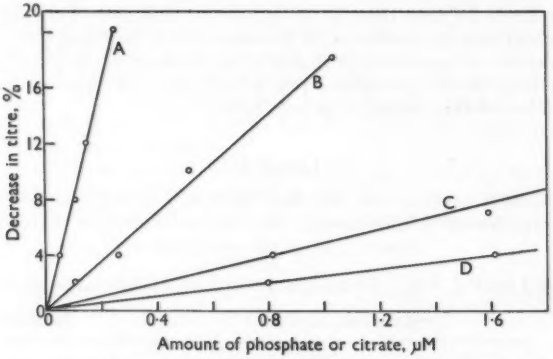

Fig. 2. Effects of phosphate and citrate in the titrations of 5 μg of calcium with murexide as indicator and 0·5 μg of magnesium with Eriochrome black T as indicator: curve A, citrate with magnesium; curve B, citrate with calcium; curve C, phosphate with calcium; curve D, phosphate with magnesium

The interfering effects of phosphate and of citric acid are shown in Fig. 2. It can be seen from Fig. 2 that 0·4 μmole of phosphate (12·4 μg of phosphorus) lowers the titrate of 5 μg of calcium by 0·1 μg or 2 per cent. and 0·8 μmole of phosphate (24·8 μg of phosphorus) lowers the titrate of 0·5 μg of magnesium by 0·01 μg or 2 per cent. This is the limit of accuracy of the titration procedure. The calcium to phosphorus ratio of $5/12{\cdot}4 = 0{\cdot}4$ may be regarded as the minimum that the murexide indicator will tolerate without yielding low values for calcium. This ratio is commonly and roughly ascertainable from food-analysis tables, and a knowledge of it may give some indication of whether "excess" of phosphate is

TABLE V

CALCIUM AND MAGNESIUM VALUES OBTAINED BY THE METHODS DESCRIBED ON A VARIETY OF ASHED AND TRICHLOROACETIC ACID EXTRACTED BIOLOGICAL MATERIALS

Material	Calcium				Magnesium			
	Solution of ash		Trichloroacetic acid filtrate		Solution of ash		Trichloroacetic acid filtrate	
	Direct, mg per 100 ml	Phosphate precipitate, mg per 100 ml	Solution of ash (direct), mg per 100 ml	Phosphate precipitate, mg per 100 ml	Direct, mg per 100 ml	Phosphate precipitate, mg per 100 ml	Solution of ash, mg per 100 ml	Phosphate precipitate, mg per 100 ml
Cow's milk	114, 112, 112	—	110, 110, 112	116, 118, 116	10·3, 10·5, 10·1	—	10·3, 9·5, 10·3	9·9, 9·7, 9·7
Hen's egg—								
Yolk	103, 99, 96	106, 103, 107	121, 107	114, 117	11·7, 9·9, 10·4	11·7, 11·6, 11·7	10·1, 11·4	11·8, 11·0
White	16·5, 14·5, 16·4	15·9, 13·9, 17·6	—	—	11·0, 11·4, 11·2	11·1, 11·1, 11·7	—	—
Skin	148, 132	135, 118	—	—	14·4, 14·3	13·6, 13·5	—	—
Shell	34·8, 34·9 (%)	36·6, 34·9 (%)	—	—	0·45, 0·75 (%)	0·45, 0·45 (%)	—	—
Bonemeal	22·3, 23·0 (%)	19·0, 21·7 (%)	—	—	0·33, 0·33 (%)	0·33, 0·31 (%)	—	—
Rat muscle	—	8·3, 6·9	—	8·3, 9·4	—	28, 29	—	31, 30
Rat kidney	—	8·6, 7·8	—	8·7, 8·5	—	23, 23	—	23, 24
Lamb's brain—								
Cerebrum	7·0, 7·2	7·2, 6·8	7·0, 7·3	6·2, 7·8	14·8, 14·4	14·8, 14·0	14·1, 14·1	14·1, 14·1
Cerebellum	—	5·9, 5·7	—	6·5, 6·5	—	13·3, 13·4	—	13·5, 14·2
Spinal bulb	5·1, 5·6	5·5, 6·0	6·3, 6·3	5·8, 6·0	13·9, 12·7	14·3, 12·7	13·2, 14·4	13·8, 14·1
Urine (not ashed)	7·3	7·8, 8·0, 7·8	—	8·0, 7·8, 8·2	3·4	3·6, 3·6, 3·5	—	3·6, 3·6, 3·7
Faeces, hospital cases—								
"*H*"	23, 24	25, 25	—	24, 23	6·3, 6·0	6·3, 5·9	—	6·0, 6·0
"*J*"	274, 274	274, 287	—	265, 269	31, 29	32, 32	—	31, 31
"*W*"	214, 225	208, 225	—	215, 215	40, 41	42, 41	—	42, 41
Oatmeal	—	62, 60	—	68, 61	—	132, 131	—	137, 135
Cocoa	93, 112	111,109	—	113, 104	446, 469	434, 447	—	446, 446
Leaves of—								
Kale	—	220, 240	—	230, 265	—	34, 33	—	33, 34
Leek	—	145, 155	—	150, 150	—	20, 22	—	18, 18
Parsley	—	170, 160	—	185, 180	—	30, 31	—	32, 34
Spinach	—	42, 42	—	44	—	39, 40	—	39
Lemon juice (not ashed)	0·0	5·2, 5·8	4·8, 5·2	2·2, 2·6	0·5	5·5, 4·9	4·9, 4·9	5·0, 4·9
Tap-water (not ashed)	11·6, 11·8	10·6, 11·2	—	—	0·45, 0·50	0·45, 0·45	—	—
Filter-paper	—	14	—	—	—	2	—	—
Oak sawdust	—	565	—	—	—	22	—	—
Anthracite coal	—	100, 117	—	—	—	37, 33	—	—

likely to be present in the material in question. Likewise the magnesium to phosphorus ratio is 0·5/24·8 = 0·02, an indication that only in exceptional circumstances is phosphate likely to affect the titration of magnesium in presence of Eriochrome black T.

Citric acid is commonly present in body fluids, bone and, especially, citrus fruits. We have observed (see Table V) that diluted lemon juice gives a zero titration for calcium and that the citric acid present prevents its complete precipitation by the phosphate method described.

Ashing of materials—

The ashing of materials has been set as an alternative to extraction and de-proteinisaton with trichloroacetic acid. For some materials, *e.g.*, bone and woody materials, ashing is almost imperative.

The subject of ashing materials for trace-element analysis has recently been one of major interest, as the recent report by Gorsuch[12] testifies. When working on a micro scale the problem of finding a satisfactory method of ashing in the determination of calcium and magnesium is similar. We have had much satisfaction when using Middleton and Stuckey's principle, and nearly all of the values for ashed materials reported in Tables II to V were obtained by the method already described.

General remarks—

No detailed discussion of the contents of Table V is necessary here. It may be noted, however, that the samples of some of the materials were not necessarily homogeneous, and some of the variations shown may thus be accounted for. Some of the values were obtained in the earlier stages of the work, and no great precision was to be expected. Fairly satisfactory values are shown for faeces, from homogenised material, from distinct pathological conditions. The degree of concordance of values obtained by the different procedures for widely different material would appear to be fair evidence for the general soundness of the phosphate precipitation method.

The reproducibility of values and precision attainable by the method may be better judged from the values in Table IV. These were obtained from dried liver powder and calculated back to wet weight.

It is concluded that the procedure of preference is phosphate precipitation of trichloroacetic acid filtrates and secondly phosphate precipitation of the solution of the ashed material. Direct titration of ashed material, especially after thiocarbamate treatment, often yields values, as shown in Table V, close to those found by the phosphate procedure; this likewise holds for acetic acid filtrates. The two last-mentioned procedures are, however, more liable to error than is the phosphate precipitation procedure. The analyst must choose the procedure best suited to the material in question.

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The Determination of Hexachloro*cyclo*hexane Residues in Foodstuffs

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A method is described for separating and determining hexachloro*cyclo*hexane residues in a variety of foodstuffs, notably, fats, oils and vegetables.

In a recent paper Sergeant and Wood[1] described a method for isolating DDT from fatty or plant materials, based on its resistance to attack by a mixture of concentrated and fuming sulphuric acids. Hexachloro*cyclo*hexane (BHC) is similarly stable to this treatment, and the application of this property to the separation of BHC residues and their determination by a suitable adaptation of Hancock and Laws's method[2] is described here. Results obtained with a variety of foodstuffs are recorded.

Several fundamental methods exist for determining BHC residues, principally those described by Phillips,[3] Bradbury and Standen[4] and Schechter and Hornstein.[5] The first method, involving reaction of the pesticide with aniline to give a mixture of products that on treatment with acidified vanadium pentoxide solution produces a violet colour, has been studied during this work, but was found to be deficient in two respects; the non-reproducibility of results and the rapid fading of the coloured complex. Bradbury and Standen's method, depending upon the production of a mixture of yellow monochlorodinitroresorcinols from BHC by successive dehydrochlorination, nitration and hydrolysis reactions, has not been investigated. Although more specific for BHC, it is said to have the disadvantage of giving a final colour having a lower optical density per microgram of the pesticide than those produced in the other available procedures. The method most widely used is that proposed by Schechter and Hornstein and adopted by the Association of Official Agricultural Chemists.[6] In this the BHC is dechlorinated by zinc and acetic acid to benzene, which is then carried over by a stream of carbon dioxide (generated from malonic acid *in situ*) into a nitration acid mixture; the *m*-dinitrobenzene so formed is isolated and yields a characteristic colour on treatment with a suitable alkali and ketone. A modification of this method has been devised in this laboratory by Hancock and Laws[2] for determining traces of BHC in water and sewage effluents. Hancock and Laws have shown that the use of a simpler apparatus consisting of a flask, a condenser and a sintered-glass bubbler is as satisfactory as the more elaborate design used by Schechter and Hornstein; it also makes possible considerable reduction in the time required for the reaction to proceed to completion.

Experimental

In the work described the size of the reaction flask has been reduced and the design of the sintered-glass bubbler modified so as to make Hancock and Laws's apparatus more suitable for residue analysis. Methylene chloride has been found more convenient than ether as an extracting solvent for the *m*-dinitrobenzene, it being non-inflammable and heavier than water. Also, the composition of the nitration acid has been changed to give a less viscous liquid and hence a more uniform bubble formation from the bubbler. Because of the relatively large amounts of BHC dealt with in residue work, the dechlorination reaction time has been increased from 1 to 1½ hours and the time taken for the colorimetric procedure has been considerably shortened by vigorously shaking the ketone - alkali solution of the *m*-dinitrobenzene.

Hexane is used in the proposed method as the extracting solvent for BHC, although previous workers[7,8] have favoured chlorinated hydrocarbons, as they are considered to be free from aromatic impurities that interfere with the BHC determination.[5] Nevertheless, hexane has the advantages of complete immiscibility with water, ready volatility and a general applicability to the extraction of BHC from plant or fatty material. The aromatic impurities in hexane, finally concentrated in the residue, were found to be removed in a stream of carbon dioxide simply by heating the residue under reflux in a glacial acetic acid solution of malonic acid before reduction by zinc and acetic acid; distillation[9] of some of

the acetic acid to carry over the impurities was found to be unnecessary. Other interfering extractives originating from the sample under test are to a considerable extent removed at the same time. No loss of BHC occurred during this operation.

Method

Apparatus—

BHC reaction flask—A round-bottomed 50-ml flask having a neck 12-cm long fitted with a B14 socket.

Condenser—A 10-cm Liebig condenser with a B14 cone at the bottom and a B14 socket at the top.

Sintered-glass bubbler—A gas-distribution tube, obtained from A. Gallenkamp & Co. Ltd. (Ref. No. 28X2), fitted with a B14 cone and bent once at right angles and twice at an angle of 135°, as shown in Fig. 1. This design facilitates the washing of the glass sinter.

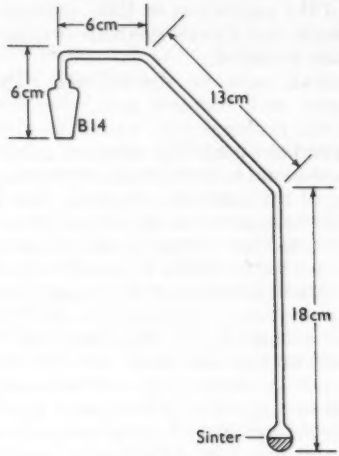

Fig. 1. Sintered-glass bubbler

Mechanical shaker.

Polytetrafluoroethylene—A small piece, 3 mm × 5 mm, has been used successfully in preventing bumping in a distilling liquid. The same piece may be re-used many times.

Glass tube to hold sodium sulphate—A tube 16 mm in bore by 150 mm long, with a 3 to 4-mm hole at the bottom.

Details of the other apparatus required, *viz*, mincing machine, extraction flasks, mechanical shaker, chromatographic tube and evaporator have been described previously.[10]

Reagents—

Nitration mixture—Mix 1 volume of fuming nitric acid with 5 volumes of concentrated sulphuric acid, both of analytical-reagent grade.

Methylene chloride—Laboratory-reagent grade.

Sodium hydroxide solution—A 10 per cent. w/v aqueous solution; it should be freshly diluted with water to 2 per cent. w/v before use.

Sodium sulphate, anhydrous—Analytical-reagent grade.

Ethyl methyl ketone—Distil through a column, and collect for use a constant-boiling fraction in the range 79° to 81° C.

Potassium hydroxide solution—A 50 per cent. w/v aqueous solution.[11]

Glacial acetic acid—Heat under reflux 500 ml of laboratory-reagent grade glacial acetic acid with 10 g of zinc for 3 hours, and distil off and discard the first 100 ml. Collect the next 350 ml for use.

Malonic acid—Fine-chemicals grade. High blank values arising from certain samples of malonic acid have been avoided by heating the finely ground acid at 90° C for 3 hours.

Zinc dust—Analytical-reagent grade.
Liquid Paraffin, B.P.
Specifications of the other reagents required, *viz*, hexane, acetone, concentrated sulphuric acid, sulphuric acid mixture, Celite and amyl alcohol have been described previously.[1]

PROCEDURE FOR EXTRACTING BHC—

The sulphuric acid treatment, already described in detail for DDT residues in various foodstuffs,[1] is also applicable to the recovery of BHC residues. A hexane extract of the sample is treated with a mixture of concentrated and fuming sulphuric acids. After separation, the hexane layer is decanted through a Celite - sulphuric acid column. The further hexane washings of the sulphuric acid layer are also passed through the column.

For the proposed application of the method collect the eluate from the column in an evaporator attached to a BHC reaction flask. Add 2 drops of liquid paraffin to the hexane extract, and evaporate to a volume of a few millilitres. Remove the last traces of solvent with a stream of dry air at 40° C.

PROCEDURE FOR TREATING BHC RESIDUE—

Add 2 g of malonic acid and 10 ml of glacial acetic acid to the residue in the reaction flask, and heat to boiling. Allow the solution to reflux at the top of the neck of the flask for 45 minutes. Remove the flask from the source of heat, and cool to room temperature; add a further 2 g of malonic acid and 1 g of zinc dust, rinsing down the neck with 2 ml of glacial acetic acid from a pipette, and wipe the flask socket clean with a piece of filter-paper.

PROCEDURE FOR DETERMINING BHC—

Assemble the dechlorination apparatus, sealing the joints with glacial acetic acid.[12] Immerse the sintered-glass bubbler in 5 ml of nitration acid contained in a test-tube. Allow the condenser water to run very slowly, so that it emerges dropwise. Heat under reflux carefully, controlling the heat to generate a steady, gentle stream of bubbles through the glass sinter. Heat the flask either directly over a luminous flame from a micro burner or on a sand-bath over a bunsen flame. With either method of heating shield the flask from draughts. After 1½ hours lower the test-tube and allow the glass sinter to drain, then rinse the latter inside and out with about 20 ml of ice-cold distilled water into a 100-ml separating funnel. Add the nitration acid to the contents of the funnel, rinsing the tube well with a further 20 ml of water. Cool, and extract by shaking for 1 minute with 40 ml of methylene chloride previously used to rinse the nitration tube. Allow the layers to separate, and run the methylene chloride into a second 100-ml separating funnel. Re-extract the aqueous layer with a further 20 ml of methylene chloride, combine the methylene chloride extracts in the second separating funnel, and wash with 25 ml of 2 per cent. sodium hydroxide solution. Run the methylene chloride through a glass tube containing 5 g of anhydrous sodium sulphate (supported by a plug of cotton-wool) into a 100-ml stoppered flask. Successively wash the two separating funnels, their contents and the sodium sulphate with the same 20-ml portion of methylene chloride, and finally wash the sodium sulphate with a further 10-ml portion. To the contents of the flask add 5 drops of liquid paraffin and a small piece of polytetrafluoroethylene; reduce the volume of solvent to 5 ml by evaporation on a steam-bath. Carefully remove the remaining solvent with a current of dry air at room temperature.

Dissolve the residue in 20 ml of ethyl methyl ketone, and, with a pipette, transfer an aliquot to another 100-ml stoppered flask. Adjust the volume to 10 ml with fresh ethyl methyl ketone, add 1 ml of 50 per cent. potassium hydroxide solution, secure the stopper, and shake vigorously in a mechanical shaker for 3 minutes. Set aside in the dark for 8 minutes to allow the colour to develop fully, and measure the optical density in a 1-cm cell at 570 mμ after a further 2 minutes. Carry out the entire procedure on the same amount of BHC-free material to ascertain the blank value of the sample.

PREPARATION OF CALIBRATION GRAPH—

Ascertain the reagent-blank value by the proposed method. The optical density should be less than 0·05.

Prepare a glacial acetic acid solution containing 20 μg of pure gamma-BHC per ml. With a pipette place aliquots of from 1 to 5 ml of this solution in the BHC reaction flask,

make up to 10 ml with glacial acetic acid, and proceed with the determination as described above. Plot the optical densities, less reagent blank, against micrograms of gamma-BHC present. An optical density of 0·0065 to 0·007 per microgram of gamma-BHC should be obtained.

TABLE I

RECOVERY OF BHC ADDED TO FOODSTUFFS

Material	Gamma-BHC Added, p.p.m.	Gamma-BHC Found, p.p.m.	Recovery, %	Apparent gamma-BHC in control material, p.p.m.
Blackcurrants (ripe) ..	10·0	10·1	101	0·09
Blackcurrants (immature) ..	1·0	0·96	96	0·33
Cabbage	1·0	0·99	99	0·08
	10·0	10·0	100	0·02
Carrot	4·0	4·05	101	0·04
	10·0	10·44	104	0·05
Wheat grain	4·0	4·0	100	0·10
	40·0	37·8	95	0·00
Tomato	1·0	1·04	104	0·07
	10·0	9·83	98	0·06
Butter	16·0	15·4	96	0·00
	40·0	37·6	94	0·00
Milk	4·0	3·77	94	0·11
	10·0	10·3	103	0·06
Linseed oil	16·0	15·8	99	0·47
	40·0	37·2	93	0·44
Olive oil	16·0	15·4	96	0·15
	40·0	40·1	100	0·09
Rape oil	16·0	14·6	91	0·21
	40·0	36·8	92	0·21

RESULTS

BHC was added to various foodstuffs; the results of analyses by the proposed method are shown in Table I. The fruit and vegetable samples used were known to be free from BHC. The history of the fatty materials was unknown. The figures for gamma-BHC found have been corrected for the apparent BHC in the control material.

I thank Mr. G. A. Sergeant for his helpful advice. The paper is published by permission of the Department of Scientific and Industrial Research.

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The Determination of Saccharin

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Saccharin can be quantitatively precipitated, as its silver salt, from solutions at pH values below 6, and this fact has been used in evolving a simple and rapid volumetric method for determining saccharin and its preparations. Sodium hydrogen carbonate and sodium cyclamate, if present, do not interfere; interference from chloride is easily overcome by a slight modification of the method.

The results are reproducible within a narrow range and similar to those obtained by standard methods.

Of the various methods[1,2,3] available for determining saccharin and its preparations, that described by Richmond and Hill[3] is the best and has been adopted by official pharmacopoeias.[4,5] Although this method is reliable, it is time-consuming and hence inconvenient for routine work.

Saccharin, being an imide, forms a silver salt analogous to the mercury salts described by Manson Auld.[6] This reaction has been studied, and a simple and rapid volumetric method for the quantitative determination of saccharin and its preparations—sodium saccharin, elixir saccharin and tablet saccharin—has been evolved on the basis of that reaction.

Method

Reagents—

All materials should be of recognised analytical-reagent grade.
Ammonia solution, 2·5 *per cent. w/w.*
Acetic acid, 10 *per cent. w/w.*
Silver nitrate, 0·1 N.
Nitric acid.
Ammonium thiocyanate, 0·1 N.
Ammonium ferric sulphate indicator solution.

Procedure for saccharin, sodium saccharin and elixir saccharin—

Accurately weigh about 0·3 g of saccharin, and dissolve in a mixture of 20 ml of water and 2 ml of 2·5 per cent. w/w ammonia solution. (For sodium saccharin, accurately weigh about 0·4 g, and dissolve in 20 ml of water, and for elixir saccharin, take 5 ml and add 15 ml of water.) Add to the solution 2 ml of 10 per cent. w/w acetic acid and then 25 ml of 0·1 *N* silver nitrate. Shake well, heat in a bath of boiling water for 2 minutes, and then cool in a bath of ice. Separate the precipitate by suction through a No. 4G ground-glass filter, and wash the flask and precipitate with three 5-ml portions of ice-cold distilled water. Acidify the filtrate with 10 ml of nitric acid, add 2 ml of ammonium ferric sulphate indicator solution, and titrate the excess of silver nitrate with 0·1 *N* ammonium thiocyanate. Carry out a blank determination by using the same amounts of reagents, but omit the sample. The difference between the two titres represents the amount of silver nitrate used. (One millilitre of 0·1 *N* silver nitrate is equivalent to 0·01832 g of saccharin or 0·02412 g of sodium saccharin.)

Procedure for tablet saccharin—

Accurately weigh a portion of powdered tablets equivalent to about 0·6 g of saccharin, transfer to a 50-ml calibrated flask with distilled water, and dilute to the mark. Shake for 5 minutes, and filter through a filter-paper. Reject the first 10 to 15 ml of filtrate, and collect the remainder. Take 25 ml of the filtrate, and continue as described under "Procedure for saccharin, sodium saccharin and elixir saccharin," beginning with "Add to the solution 2 ml of 10 per cent. w/w acetic acid. . . ."

Discussion of results

Samples of Saccharin B.P., Sodium saccharin B.P. and Elixir saccharin B.P.C. from reputable manufacturers were analysed by the proposed method and found to contain

98·49, 99·89 and 7·17 per cent. of saccharin, respectively, each result being the mean of three determinations. These results were reproducible and similar to those found by standard methods[4,5] (98·89, 99·72 and 7·20 per cent.).

EFFECT OF SODIUM HYDROGEN CARBONATE—

Sodium hydrogen carbonate is a common constituent of some saccharin tablets, and, although the 2 ml of 10 per cent. w/w acetic acid used are sufficient for the amount usually present, a study was made to ascertain the optimum pH at which saccharin was quantitatively precipitated. A 0·3-g portion of saccharin was analysed, different amounts of 10 per cent. w/w acetic acid being used. In each experiment the pH was measured with a Cambridge direct-reading pH meter after the solution had been heated in a water bath and then cooled to room temperature. The recoveries of saccharin, each being the mean of two determinations, were—

Amount of 10 per cent. w/w acetic acid added, ml ..	0·0	0·25	0·5	0·75	1·0	1·5	2·0	3·0	4·0
pH	8·3	6·9	6·6	6·1	5·2	4·4	4·0	3·9	3·8
Recovery, %	95·37	97·12	98·28	98·43	98·28	98·58	98·58	98·43	98·58

These results show that the recovery of saccharin at pH values below 6 was practically constant; it is therefore suggested that the pH of the solution be adjusted to less than 6 by adding the necessary amount of 10 per cent. w/w acetic acid.

EFFECT OF SODIUM CYCLAMATE—

Sodium cyclamate is also a constituent of some tablets, but does not interfere with the proposed method. This was established by separately mixing 0·3-g portions of saccharin with 0·1, 0·3 and 0·5 g of sodium cyclamate N.N.D. (Sansugar) and analysing the mixtures. The recoveries of saccharin were 98·28, 98·28 and 98·58 per cent., respectively, each result being the mean of two determinations.

EFFECT OF CHLORIDE—

Chlorides, if present, will interfere, but are easily dealt with by dissolving the precipitate in warm diluted nitric acid. To study this, separate mixtures of saccharin and sodium chloride were analysed, 50 ml of 0·1 *N* silver nitrate being added in order to leave a sufficient excess for co-precipitating chlorides. In each experiment the washed residue in the filter was dissolved by treatment with four 10-ml portions of warm 50 per cent. v/v nitric acid, and the resulting solution was titrated with 0·1 *N* ammonium thiocyanate, ammonium ferric sulphate solution being used as indicator. This titre represented the amount of silver nitrate that had combined with saccharin. The samples used consisted of 0·3-g portions of saccharin mixed with 0·1- and 0·15-g portions of sodium chloride; the recoveries of saccharin, each being the mean of two determinations, were 98·03 and 98·13 per cent., respectively.

The results above show recoveries of saccharin between 98 and 98·6 per cent. This is because the saccharin used had an average purity of 98·49 per cent. by the proposed method and 98·89 per cent. by the method recommended in the British Pharmacopoeia.

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The Determination of Calcium and Magnesium in Waters by Automatic Titration

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After preliminary work on a back-titration procedure, a direct method for determining both calcium and magnesium in the same sample of water has been worked out. After acidification, the sample is boiled to remove carbon dioxide and then cooled. An appropriate volume of sodium hydroxide solution is then added, together with a known volume of a standard mercuric solution. The calcium is then titrated with a standard solution of ethylenediaminetetra-acetic acid in an automatic recording titrimeter, a bridged calomel reference electrode and a mercury-plated silver indicator electrode being used. The solution is then acidified and is made appropriately alkaline with ammonia solution, and the titration with ethylenediaminetetra-acetic acid is continued to give a measure of the total calcium *plus* magnesium present.

The original purpose of the work described was to try to adapt the work of Khalifa, Patsak and Doppler[1] to the determination, by automatic titration, of the calcium and magnesium hardness of raw waters, boiler waters and other industrial waters. Some time ago, Haslam and his co-workers had shown that bivalent metals, such as copper, mercury, lead, zinc, etc.,[2,3] could be determined by automatic titration; the procedure involved the maintenance of a solution at pH 5·0 while it was titrated with a standard solution of ethylenediaminetetra-acetic acid (EDTA) until further addition caused no change in pH. This method as it stood could not be adapted to the corresponding determinations of calcium and magnesium, because the complexes formed by these metals with EDTA were not stable at pH 5·0. Recently, however, Khalifa, Patsak and Doppler showed that, if calcium and magnesium are complexed at high pH values, the excess of complexing agent can be readily determined by titrating back with standard mercuric nitrate solution.[1] A silver-amalgam indicator electrode and a calomel reference electrode connected to the solution being tested by a saturated ammonium nitrate bridge were used; this permitted calcium and magnesium to be determined either alone or when mixed.

Attempts to put the principle of Khalifa and his co-worker's titration method on an automatic basis, however, were not at first successful, mainly because of the lack of information about a stable bridged calomel electrode. However, with the co-operation of Mr. G. P. Alcock (Imperial Chemical Industries Ltd., Alkali Division), such a stable system was provided, and as a result we were able to establish a method for determining total calcium *plus* magnesium in various waters by automatic titration.

Back-titration method

Apparatus—

Automatic titrimeter—We used the automatic titrimeter manufactured by Electronic Instruments Ltd., although we see no reason why other automatic instruments should not be used.

Reference electrode—This electrode is shown in Fig. 1. The inner saturated-calomel reference electrode used was obtained from Electronic Instruments Ltd. This electrode rested on the rim of the outer bridge sleeve, which was three-quarters filled with a saturated solution of potassium nitrate. This bridge solution should be renewed weekly.

Mercury - silver amalgam indicator electrode—This electrode was prepared by fusing a silver wire to form a ball of silver $\frac{3}{16}$ inch in diameter at the end, cleaning the wire and mounting it in a polythene sleeve with the ball extending $\frac{1}{2}$ inch from the end. The amalgam coating was formed by immersing the clean electrode in pure mercury for 3 hours and then washing with water. The electrode attains stable characteristics and sensitivity of response after it has been used in one or two titrations.

REAGENTS—

EDTA solution, 0·004 M—Prepare this solution by accurately diluting a 0·1 *M* stock solution of EDTA made by dissolving 37·23 g of EDTA (the disodium salt of Sequestric acid, obtained from Hopkin and Williams Ltd.) in distilled water and diluting to 1 litre.

Zinc solution, 0·004 M—Prepare this solution by accurately diluting a standard 0·1 *M* stock solution of zinc. The stock solution can be conveniently prepared by dissolving the calculated amount of AnalaR zinc sulphate, $ZnSO_4.7H_2O$, previously standardised gravimetrically by the 8-hydroxyquinoline procedure, in distilled water and diluting to 1 litre.

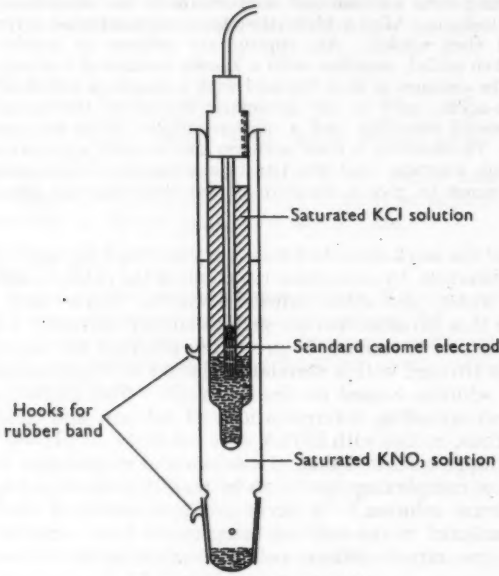

Fig. 1. Reference electrode

Mercuric nitrate solution, 0·004 M—Dissolve 1·38 g of mercuric nitrate, $Hg(NO_3)_2.H_2O$ (obtained from the British Drug Houses Ltd.), in distilled water containing sufficient nitric acid to give a clear solution, and dilute to 1 litre.

Buffer solution, pH 11—Prepare by mixing 100 volumes of *M* ammonium hydroxide with 5 volumes of *M* ammonium nitrate.

Hydrochloric acid, approximately N.

Phenolphthalein indicator solution.

PROCEDURE—

Transfer the appropriate volume of sample, as indicated below, to the titration vessel; if only a 10- or 20-ml sample is required, dilute to 100 ml with distilled water.

Hardness, as $CaCO_3$, p.p.m. w/v	0 to 3	3 to 10	10 to 30	30 to 100	100 to 350
Volume of sample, ml	400	200	100	20	10

Add a few drops of phenolphthalein indicator solution, and, should the solution turn pink, decolorise by adding *N* hydrochloric acid from a dropping-bottle to bring the pH of the solution to approximately 8·5. For each 100 ml of solution add 10 ml of buffer solution, and then add 10·0 ml of 0·004 *M* EDTA solution from a burette or pipette. Switch on the stirrer, and adjust the titrimeter for a titration with the "function" switch at mV RISING and for delivery of titrant at the fast rate up to the end-point setting of −75 mV. (This end-point potential difference has been found by preliminary manual plots of similar titrations

to be suitable for zinc, calcium, magnesium and EDTA (see Fig. 2) and in our experience is constant for a given electrode pair under the conditions of the test).

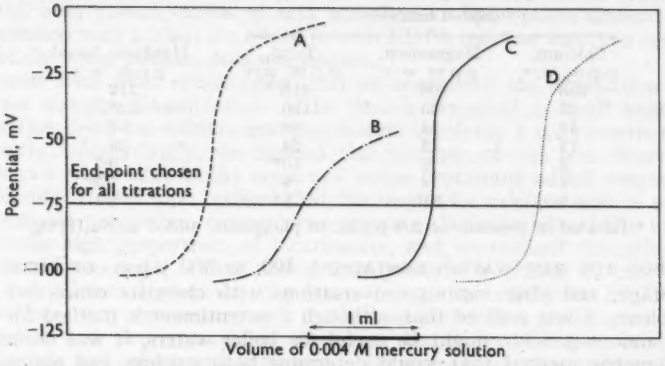

Fig. 2. Titration curves for various solutions: curve A, EDTA; curve B, magnesium and excess of EDTA; curve C, calcium and excess of EDTA; curve D, zinc and excess of EDTA

With the automatic burette filled with 0·004 *M* mercuric nitrate, switch the titrimeter to automatic titration; the mercury solution will then be delivered at the fast rate and will slightly overshoot the end-point setting before the burette valve closes. Leave the instrument switched on, and add a further 2·0 ml of 0·004 *M* EDTA solution; the burette valve will then automatically re-open and the titration will proceed at the slow drop-rate to the pre-set end-point. Read the volume of mercuric nitrate solution used. Replace the sample solution by distilled water, carry out a blank titration, and hence calculate the "usage" due to the hardness in the sample solution. In our experience this blank titre is virtually constant for each batch of solutions and need only be checked weekly.

1 ml of 0·004 *M* mercuric nitrate ≡ 0·0004 g of hardness, as $CaCO_3$.

NOTE—Should a sample of water having unknown history be received for examination and no information on the probable hardness level be available, the procedure described below can be adopted to determine the approximate hardness level; if necessary, a repeat test, as described above, can then be carried out on an appropriate volume of water.

Transfer 10 ml of sample to a 250-ml beaker, add 10 ml of buffer solution, and dilute to 100 ml with distilled water. Add 0·004 *M* EDTA solution from a burette until the titrimeter indicates that the end-point potential (−75 mV) has been reached. Continue to add EDTA solution until at least a 4-ml excess is present, and note the total volume added. Titrate this excess with 0·004 *M* mercuric nitrate as described above, and hence calculate the hardness level of the water.

STANDARDISATION OF MERCURIC NITRATE SOLUTION—

The factor for the 0·004 *M* mercuric nitrate should be checked weekly as described below.

Transfer 10·0 ml of standard 0·004 *M* zinc solution to a 250-ml beaker, add 20·0 ml of 0·004 *M* EDTA solution and 10 ml of buffer solution, and dilute to 120 ml with distilled water. Titrate the excess of EDTA solution automatically, as described above, with the 0·004 *M* mercuric nitrate (titre *A*), and carry out a blank titration omitting only the zinc solution (titre *B*).

$$\text{Factor for 0·004 } M \text{ mercuric nitrate} = \frac{10{\cdot}0}{B - A}.$$

RESULTS—

When applied to solutions containing known amounts of calcium and magnesium, as calcium chloride and magnesium sulphate, in distilled water, the procedure just described gave excellent results. Further, when applied to boiler waters it gave good replicable results in fair agreement with those obtained by the EDTA colorimetric-end-point method. The addition of phosphate to the solution before titration did not affect the test, as is shown by some typical results in Table I.

TABLE I

HARDNESS FOUND BY THE BACK-TITRATION METHOD
Figures for hardness are expressed as $CaCO_3$

Added hardness			
Calcium, p.p.m. w/v	Magnesium, p.p.m. w/v	Total, p.p.m. w/v	Hardness found, p.p.m. w/v
108	108	216	212
65	65	130	126
44	44	88	87
12	12	24	23
8	2	10*	9·7
4	4	8	7·9
1	Nil	1*	1·1

* Titrated in presence of 200 p.p.m. of phosphate, added as Na_2HPO_4.

DIRECT METHOD FOR RAW WATERS CONTAINING 100 TO 300 p.p.m. OF TOTAL HARDNESS

At this stage, and after various conversations with chemists concerned with water-softening problems, it was realised that, although a potentiometric method for determining total calcium and magnesium might be useful for boiler waters, it was desirable to work out a potentiometric method that would determine both calcium and magnesium in the same sample of an industrial raw or process water. In many ways this suggestion was helpful. We began to think in terms of the determination of (*a*) total calcium hardness and (*b*) total calcium *plus* magnesium hardness in the same sample of water by direct titration with EDTA solution. Moreover; by this time development work had led to the introduction of a simple form of a full-scale recording automatic titrimeter.[4] It seemed possible, therefore, that we might be able to use this titrimeter for a relatively simple direct titration with EDTA solution. We hoped to avoid the use of mercuric nitrate as titrant, since we believed that there might be some objection to its use in practice. We decided to try the electrode system described on p. 27 for the direct titration of calcium in presence of magnesium with EDTA solution in a sodium hydroxide medium, both with and without a trace of mercuric ions in the test solution. As can be seen from the recorded titration curves in Fig. 3, the presence of mercuric ions greatly increased the sensitivity of end-point detection, and a trace of mercuric solution was therefore added in all subsequent work.

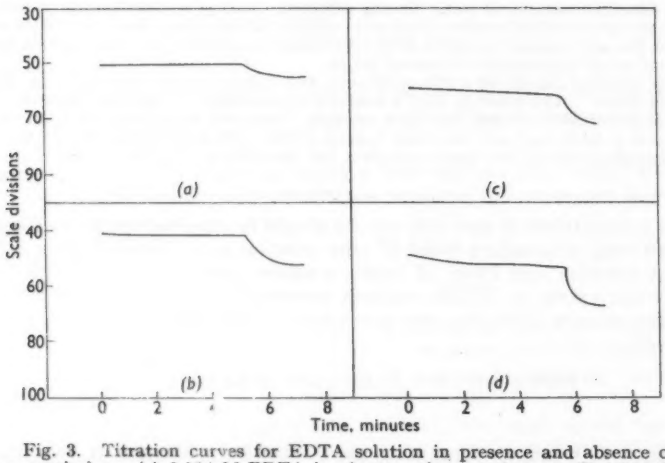

Fig. 3. Titration curves for EDTA solution in presence and absence of mercuric ions: (*a*) 0·004 *M* EDTA in absence of mercuric ions; (*b*) 0·004 *M* EDTA in presence of mercuric ions; (*c*) 0·02 *M* EDTA in absence of mercuric ions; (*d*) 0·02 *M* EDTA in presence of mercuric ions

Having decided on this system, many combinations of acid solutions of calcium and magnesium salts were then examined by a relatively simple procedure. The acid solution

containing the calcium and magnesium, with a trace of added mercuric ions, was made appropriately alkaline with sodium hydroxide solution, and the calcium was then titrated with EDTA solution in the full-scale titrimeter. When the record showed that the calcium end-point had been passed, excess of acid and then an appropriate amount of ammonium hydroxide solution were added; the titration with EDTA solution was then continued to give a measure of the total calcium *plus* magnesium.

The results were most encouraging, and we considered the application of the test to industrial raw waters. Examination of the British Standard method[5] indicated that, for colourless waters, tests for calcium and magnesium involving a colorimetric end-point were applied directly. Accordingly, we applied the principle of our test directly to Welwyn Garden City raw water and to the same raw water containing added magnesium, as magnesium sulphate. It was soon realised that the results for calcium were in error, especially when a reasonable magnesium content was present. Welwyn water, like many industrial waters, contains high proportions of bicarbonate, and we realised that direct addition of sodium hydroxide to such waters would not produce a simple system of magnesium hydroxide and calcium hydroxide, the latter being titratable with EDTA solution. It seemed, therefore, that a necessary step in the test would be a preliminary acidification of the test solution and then removal of all carbon dioxide by boiling before the addition of sodium hydroxide and subsequent titrimetry. By using this procedure we obtained correct results for the calcium and magnesium contents of the many samples tested. Failure to break down bicarbonates may lead to serious errors, as can be seen from the titration curves shown in Fig. 4, which were plotted both with and without breakdown of added bicarbonate equivalent to the calcium and magnesium in the same sample of slightly acid water. We suspect that many colorimetric difficulties hinted at in the literature may be ascribed to this cause.

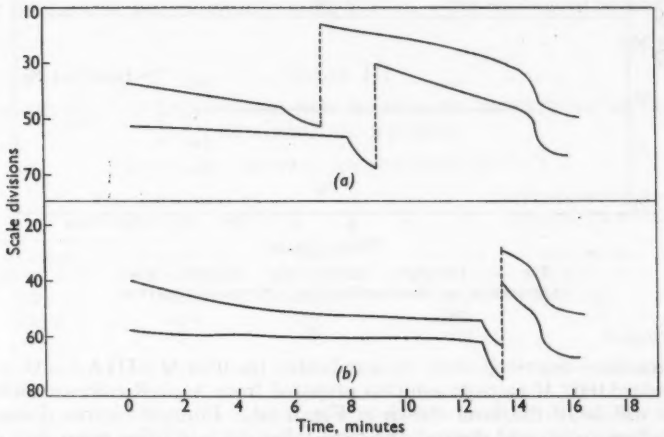

Fig. 4. Titration curves for a sample of slightly acid water: (*a*) titrated direct; (*b*) titrated after addition of hydrochloric acid and removal of carbon dioxide by boiling

APPARATUS—

The automatic recording titrimeter used in this work has been described previously.[4] The only modification was that the injector motor and chart drive were operated by a synchronised switch. The titrimeter was adjusted to give full-scale deflection at 200 mV, a chart speed of 1 inch per minute and an injection speed, for a 20-ml syringe, of 1 inch in 10 minutes. The electrode pair used in conjunction with the pH meter was again that described on p. 27.

REAGENTS—

EDTA solution, 0·02 M—Prepare by accurate dilution of a 0·1 *M* solution.
Mercuric nitrate, 0·004 M.
Ammonium hydroxide, M.

Sodium hydroxide, N.
Hydrochloric acid, N.
Phenolphthalein indicator solution.

PROCEDURE—

Place 100 ml of sample in a 250-ml beaker, and add 2 drops of phenolphthalein indicator solution. Add a 2-ml excess of *N* hydrochloric acid, and boil for 5 minutes to expel carbon dioxide. Cool, add *N* sodium hydroxide until an alkaline reaction is obtained, and then add 4 ml of alkali in excess. Transfer the beaker to the automatic recording titrimeter, add 0·2 ml of 0·004 *M* mercuric nitrate, and titrate with 0·02 *M* EDTA solution until the record shows that the calcium end-point has just been passed. Switch off the injector motor and chart drive, add *N* hydrochloric acid until an acid reaction is obtained, and then add 1 ml of acid in excess. Add 10 to 15 ml of *M* ammonium hydroxide, and allow the instrument to stabilise for 1 minute. Switch on the injector motor and chart drive, and allow the titration to proceed until the end-point for the titration of both calcium and magnesium is passed. The titration curves will be of the form shown in Fig. 5 (*b*).

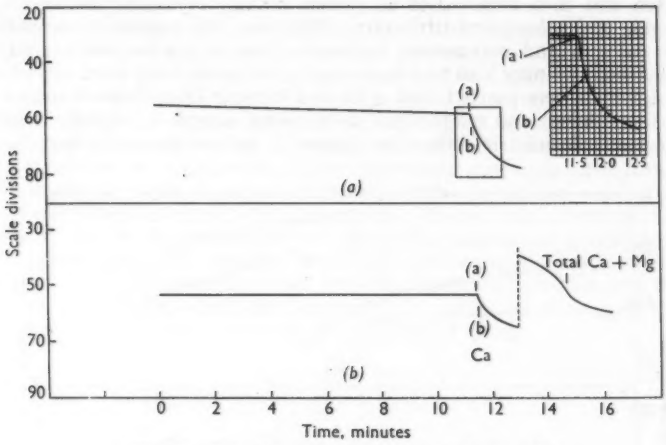

Fig. 5. Titration curves for calcium *plus* magnesium: (*a*) standardisation; (*b*) typical titration

STANDARDISATION—

Use the procedure described above to standardise the 0·02 *M* EDTA solution by titrating 10 ml of a standard 0·02 *M* calcium solution prepared from AnalaR calcium carbonate. The titration curve will be of the form shown in Fig. 5 (*a*). From the curve deduce the point of maximum inflexion (b), and convert the time taken to reach this point into millilitres of EDTA solution. In practice, we have arithmetically computed this point of maximum inflexion. For example, in the enlarged portion of Fig. 5 (*a*), the changes in e.m.f. difference (scale divisions) for each 0·1 minute from point (a) onwards are 3, 5 and 2, and maximum inflexion is therefore taken as being at 11·64 minutes. Deduce the calcium content of the sample being tested by reference to its titration curve (Fig. 5 (*b*)), calculating the amount of EDTA solution used to reach point (b) on the curve and using the factor obtained in the standardisation for the EDTA solution.

In exceptional instances, when the magnesium content is extremely low, the calcium and total-hardness end-points are so close together that it is impractical to continue the calcium titration to the point of maximum inflexion; for such titrations we have found it useful to make the appropriate calculations by using the end-points marked (a) on both sample and standardisation curves. There is no difficulty with the calcium *plus* magnesium end-point, since the point of maximum inflexion is used in the calculation of both standard and sample.

RESULTS—

The results obtained when the method was applied to mixtures containing known amounts of calcium and magnesium in distilled water, to industrial raw water and to industrial raw water containing added magnesium are shown in Table II.

TABLE II

HARDNESS FOUND BY DIRECT TITRATION

Figures for hardness are expressed as $CaCO_3$

	Added hardness			Hardness found		
	Calcium, p.p.m. w/v	Magnesium, p.p.m. w/v	Total, p.p.m. w/v	Calcium, p.p.m. w/v	Magnesium, p.p.m. w/v	Total, p.p.m. w/v
Distilled water	150	100	150	150·4	101·4	251·8
				150·1	101·7	251·8
	200	50	250	200·6	50·6	251·2
				200·6	50·6	251·2
	220	30	250	219·4	31·8	251·2
				218·7	32·7	251·4
	240	10	250	240·5	9·0	249·5
				240·5	8·7	249·2
Welwyn raw water	..	..	..	286·5	9·5	296·0
				285·9	9·9	295·8
Welwyn raw water + 50 p.p.m. of added magnesium			..	285·6	60·2	345·8
				285·6	61·1	346·7

The method was also applied to samples of water from other parts of the country. These samples were kindly sent to us by Mr. K. B. Coates, who had previously examined them by conventional colorimetric procedures involving EDTA. The two sets of results are compared in Table III.

TABLE III

COMPARISON OF RESULTS FOR RAW WATERS BY POTENTIOMETRIC AND COLORIMETRIC METHODS

Figures for hardness are expressed as $CaCO_3$

Sample No.	Hardness found by potentiometric method			Hardness found by colorimetric method		
	Calcium, p.p.m. w/v	Magnesium, p.p.m. w/v	Total, p.p.m. w/v	Calcium, p.p.m. w/v	Magnesium, p.p.m. w/v	Total, p.p.m. w/v
1	197	184·5	381·5	202	177	379
2	365·5	15·5	381	367	11	378
3	53	12	65	53	9	62

INTERFERENCE—

As the direct method might be used in practice for the titration of calcium and magnesium in solutions containing small amounts of other metals, such as copper, iron, etc., the effect of these metals was investigated. A solution containing 200 p.p.m. of calcium hardness and 53 p.p.m. of magnesium hardness, *i.e.*, a total hardness of 253 p.p.m., as $CaCO_3$, was titrated in presence of 10 p.p.m. of each added metal to both the calcium and the calcium *plus* magnesium end-points. The results are shown in Table IV.

Iron appears to cause negligible interference at both calcium and total-hardness end-points. Copper, zinc, manganese and nickel appear to cause no interference at the calcium end-point, but are titrated at the total-hardness end-point. Aluminium does not cause interference at the calcium end-point, but is not completely titrated at the total-hardness end-point.

The presence of 10 p.p.m. of phosphate, as PO_4^{3-}, did not affect either end-point; 200 p.p.m. of orthophosphate masked the calcium end-point, but did not affect the total-hardness end-point. The presence of 10 p.p.m. of sodium hexametaphosphate did not apparently affect either end-point.

TABLE IV

EFFECT OF VARIOUS METALS

Figures for hardness are expressed as $CaCO_3$. Ten parts per million of each metal were added

Metal added	Calcium hardness equivalent to added metal, p.p.m. w/v	Hardness found			Theoretical hardness if all added metal were titrated, p.p.m. w/v
		Calcium, p.p.m. w/v	Magnesium, p.p.m. w/v	Total, p.p.m. w/v	
None	Nil	200	53	253	253
Iron	17·9	198	—	255	271
Copper	15·7	201	—	269	269
Zinc	15·3	201	—	273	268
Nickel	17·0	200	—	272	270
Manganese	18·2	199	—	268	271
Aluminium ..	37·1	199	—	269	290

DIRECT METHOD FOR WATERS CONTAINING UP TO 10 p.p.m. OF TOTAL HARDNESS

The application of the direct method to the examination of waters having an extremely low hardness, *e.g.*, boiler waters, was considered, and it was found that, when a total-hardness figure only was required, the method could be applied directly to the water in the way described below.

PROCEDURE FOR DETERMINING TOTAL HARDNESS—

Transfer 400 ml of sample to a 600-ml beaker, and neutralise to phenolphthalein with *N* hydrochloric acid. Add 40 ml of *N* ammonium hydroxide, and titrate directly in the automatic recording titrimeter with 0·004 *M* EDTA solution until the end-point for calcium *plus* magnesium is passed. Standardise the 0·004 *M* EDTA solution by titrating under the same conditions 5 ml of standard 0·004 *M* calcium solution prepared from AnalaR calcium carbonate. Calculate the total-hardness content of the water.

When the effect of interfering ions in this test was investigated, 10 p.p.m. of iron, 100 p.p.m. of aluminium and 300 p.p.m. of phosphate appeared to exert a negligible effect in the titration of water containing 6 p.p.m. of calcium and 3 p.p.m. of magnesium.

PROCEDURE FOR DETERMINING CALCIUM AND MAGNESIUM HARDNESS—

On rare occasions it may be necessary to determine both calcium hardness and magnesium hardness, as distinct from total calcium *plus* magnesium hardness in such a water. For these determinations we find that it is probably desirable to carry out a preliminary evaporation of the sample, with subsequent ashing of the evaporation residue to destroy organic matter, before the titration procedure detailed for raw waters is applied. A suitable procedure is summarised below.

Evaporate 400 ml of sample to dryness, preferably in a platinum basin, gently ignite the residue, and evaporate the ash to dryness three times with a small amount of hydrochloric acid. Dissolve the residue in hot water, and filter the solution into a titration beaker. Repeat the ignition and evaporation with any insoluble matter remaining on the filter-paper.

Dilute the filtrate to 100 ml, add 0·2 ml of 0·004 *M* mercuric nitrate and then a 4-ml excess of *N* sodium hydroxide. Titrate to the calcium end-point with 0·004 *M* EDTA solution.

After the calcium end-point has been passed, acidify the solution with hydrochloric acid, and add 15 ml of *N* ammonium hydroxide. Allow the instrument to stabilise, and proceed with the titration to the total-hardness end-point.

Correct the volume of titrant delivered at the calcium and total-hardness end-points for the 0·2 ml of 0·004 *M* mercuric nitrate added to improve the sensitivity of the end-points, and calculate the respective hardness values.

Note that, when the titration curves indicate that all the hardness is due to calcium, it is desirable to repeat the determination with a known amount of added magnesium, which should of course be found in the final titrimetric test. Corresponding remarks apply when the

hardness is wholly due to magnesium, *i.e.*, a repeat determination should be carried out in the presence of added calcium.

So far as we are aware, the proposed method is the first in which calcium and magnesium are directly titrated potentiometrically with standard EDTA solution.

We thank Mr. G. P. Alcock for details of the reference electrode used, and Messrs. J. Edwards and K. B. Coates for many helpful discussions and various samples used in testing the methods.

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The Electrometric Determination of Dissolved Oxygen in Aqueous Solution

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A method depending on the reactivity of chromous ions towards oxygen is described. The chromous reagent is electrolytically prepared in the solution being investigated, and the apparatus immediately detects the excess of chromous ions marking the end-point of the coulometric titration. The method is straightforward, and results are accurate to within less than 2 per cent. at concentrations as low as three parts of oxygen per hundred million parts of water.

Because of the significance of trace amounts of oxygen, methods for its determination at great dilution have been accorded much attention. Various modifications of Winkler's method[1] seem still to be the most popular and are essentially based on—

(*i*) evolution of the dissolved oxygen, which is subsequently "fixed" and determined in absence of interfering substances[2];

(*ii*) separate determination of the magnitude of the errors involved, these errors being discounted by using correction factors (as described in an unpublished report to the Joint Research Committee on Boiler Feed-Water Studies, May, 1953);

(*iii*) double titrations involving a blank procedure to remove errors.[3,4]

We have made use of the reaction between oxygen and chromous ions, which are highly efficient in removing oxygen from a stream of carrier gas.[5] A chromous reagent has previously been used in the determination of dissolved oxygen.[6]

The electrolytic reduction of chromic ions to the chromous state at lead electrodes has been investigated, and conditions have been found in which efficiencies as high as 85 per cent. can be attained.[7] Complete reduction of dichromate at amalgamated-lead cathodes has been achieved, but the current efficiency was not stated.[8]

Experimental

Apparatus—

Titration cells—Relatively concentrated solutions were titrated conventionally in the cell shown in Fig. 1 (*a*); more dilute solutions were titrated coulometrically in the cell shown in Fig. 1 (*b*).

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Constant-current source—An electronically controlled constant-current source of the type described by Carson[9] was completely satisfactory. The value of the current was found by measuring potentiometrically the voltage drop across a standard 100-ohm resistor.

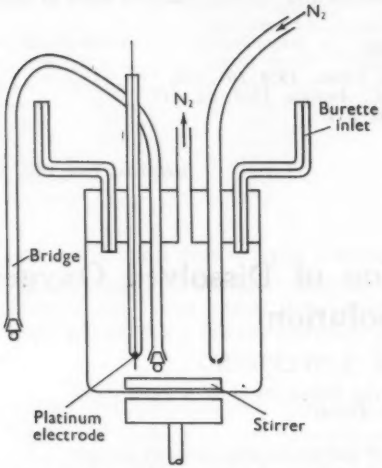

Fig. 1 (*a*). Cell used for conventional titrations

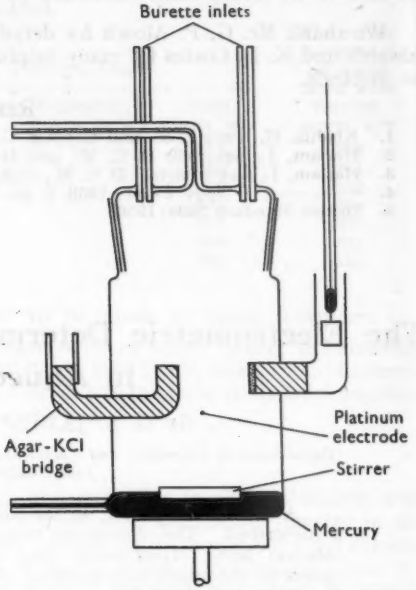

Fig. 1 (*b*). Cell used for coulometric titrations

REAGENTS—

Purified nitrogen—Purified nitrogen was used for the removal of dissolved oxygen from aqueous solutions. In order to remove traces of oxygen, commercial nitrogen was passed through a series of three scrubbing towers containing alkaline pyrogallol and then through one tower packed with amalgamated zinc and filled with 0·3 *N* chromous sulphate in 2 *N* sulphuric acid. Finally, the gas was washed by passing it through distilled water.

Chromic sulphate solution, air-free—A solution was prepared from analytical-reagent grade chromic sulphate hexahydrate and, when freed from dissolved oxygen, was stored under positive nitrogen pressure in an apparatus of the type described by Stone.[10] Oxygen was removed by inverting the apparatus and evaporating at least one-fifth of the water, at the same time bubbling a stream of purified nitrogen through the boiling solution. The apparatus was then closed to the atmosphere and allowed to absorb nitrogen as it cooled to 0° C, at which temperature it was closed to nitrogen and re-inverted. When the ambient temperature was reached, the air-free reagent was stored under a considerable pressure of nitrogen and was used via the burette forming part of the apparatus.

Acid chromous sulphate reagent solution—An approximately 0·02 *M* solution of this reagent was prepared from pure potassium dichromate as described by Lingane and Pecsok.[11] A platinum - standard calomel electrode indicator system was used to standardise the solution against 0·02 *M* cupric sulphate in accordance with the equation—

$$2CrSO_4 + 2CuSO_4 \longrightarrow Cu_2SO_4 + Cr_2(SO_4)_3.$$

The titration was carried out in the cell shown in Fig. 1 (*a*). The cupric sulphate solution was placed in the cell by pipette, and oxygen was removed by bubbling purified nitrogen through the stirred solution for 15 minutes. The solution was then titrated through one of the burette inlets. The results were extremely reproducible and indicated that this method of oxygen removal was adequate when analysing these relatively concentrated solutions.

Standard oxygen solutions—Aqueous solutions containing known amounts of oxygen, as small as 1×10^{-5} g per ml, were prepared as described by James and Murray.[12] Subsequent

dilution in the titration cell gave solutions containing down to three parts of oxygen per hundred million parts of solution for analysis. The accuracy of this method for preparing standard solutions of gases has been well established. (See also Table I).

END-POINT PROCEDURE—

As the chromic - chromous couple is incompletely reversible, the use of polarised inert electrodes for end-point indication was relatively satisfactory only in the presence of high concentrations of chloride ion, when the reversibility of the couple improves.

Chromous ion in potassium chloride solution, however, yields a well defined polarographic wave at an anodic half-wave potential of about −0·4 volt and its presence is therefore detectable by an amperometric procedure. The indicator circuit used in these determinations was based on this premise and consisted of a galvanometer in series with the platinum indicator electrode and a saturated-calomel electrode. It also incorporated a conventional potential-divider circuit by means of which an opposing potential could be applied to the two electrodes. Electrical connection between the cell solution and the standard calomel electrode was established via an agar-saturated potassium chloride bridge.

If, before the end-point, no potential was impressed on the indicator circuit, a variable current of maximum value about 0·5 μA flowed in the circuit, owing to reduction of oxygen. By impressing 0·3 volt on the electrodes—platinum positive—this maximum value could be reduced to approximately 0·05 μA, and the error thereby introduced into the titration results diminished to negligible proportions.

A diffusion current in the opposite sense to that just described was detectable as soon as an excess of chromous ions had been generated. The steadiness of all diffusion currents was dependent on adequate stirring of the solution.

To test this end-point procedure, water that had been saturated with oxygen and then exposed to the atmosphere for 2 hours was titrated in the cell shown in Fig. 1 (*a*). The cell was thoroughly flushed with purified nitrogen before the introduction of the solution. In Table I, the results of these titrations are compared with those found by Winkler's method on the same solutions. In each titration, the solution was made *M* in potassium chloride to prevent the setting-up of migration currents in the indicator circuit.

TABLE I

COMPARISON OF RESULTS BY THE PROPOSED METHOD AND WINKLER'S METHOD
In each experiment 40 ml of water were titrated

Concentration of chromic ions, *M*	Acidity		Volume of 0·02 *M* chromous chloride used, ml	Oxygen found, at N.T.P.—	
	pH	as sulphuric acid, *N*		by proposed method, ml per litre	by Winkler's method, ml per litre
—	6	—	2·033	5·285	5·2
—	4	—	2·045	5·318	5·30
—	2	—	2·062	5·362	5·35
—	—	3	2·051	5·333	5·31
0·2	6	—	2·080	5·410	5·40
	4	—	2·021	5·255	5·26
	2	—	2·030	5·275	5·28
	—	3	2·056	5·340	5·35

As it was intended to prepare the chromous reagent coulometrically, a check on the effects of chromic ion concentration and pH on the end-point was incorporated in this series of experiments. As shown by Table I and Fig. 2, the accuracy and sharpness of the end-point were not appreciably altered by limited variation in either of these factors.

ELECTROLYTIC PREPARATION OF CHROMOUS IONS—

Preliminary experiments with a lead cathode and platinum anode for the electrolysis of chromic solutions having various concentrations and pH values showed that chromous ions could be produced with constant current efficiency at cathode potentials below 0·73 volt against a saturated-calomel electrode. However, if the lead electrode was left standing in the solutions, its surface rapidly deteriorated, and lead was consequently abandoned in favour of mercury, which has a higher hydrogen overvoltage.

In connection with the proposed use of mercury as generator cathode, a study of the

polarographic behaviour of chromic sulphate in 0·1 *M* potassium chloride was useful. The chromic ion undergoes stepwise reduction at a dropping-mercury electrode. The half-wave potential of the first step, corresponding to the reduction of chromic to chromous ion, occurs at −0·9 volt, and the second half-wave potential, corresponding to the reduction of chromous ion to the metal, occurs at about −1·5 volts, both potentials being measured against a saturated-calomel electrode. The polarographic behaviour is sensitive to changes in pH. At pH 1·5, the second step fails to develop and at pH 6 the first wave is poorly developed. An ideal polarogram is obtained between pH 3 and 4, the latter being the natural pH of 0·001 *M* chromic sulphate in 0·1 *M* potassium chloride. From these figures it was inferred that when a mercury cathode was used at potentials between −0·8 and −1·2 volts in solutions having pH values between 3 and 4 it was possible to produce chromous ions with 100 per cent. current efficiency. No specific experiment was performed to prove this conclusion, but its validity was inferred from the subsequent results of coulometric titrations of standard oxygen solutions.

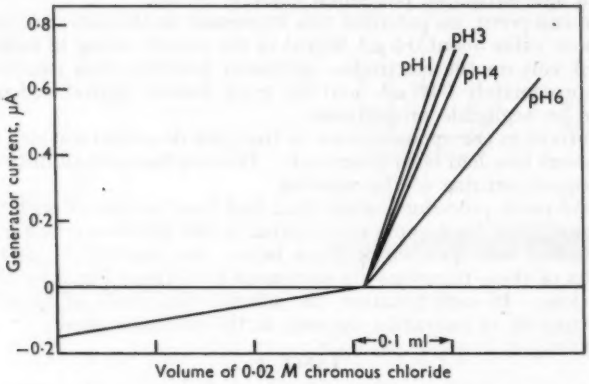

Fig. 2. Graphs showing accuracy and sharpness of end-points

COULOMETRIC TITRATIONS—

For these titrations the cell shown in Fig. 1 (*b*) was used. It was first flushed with purified nitrogen, which, in turn, was completely displaced by mercury. With the tap on the outlet capillary closed, the water sample and then air-free chromic chloride solution could be drawn into the cell through the appropriate burette inlet by lowering the mercury reservoir. A pool of mercury connected to the generator circuit through the reservoir was left in the cell to act as generator cathode. The generator anode was approximately 1 sq. cm of platinum foil in saturated potassium chloride solution; it was separated from the contents of the cell by a sintered-glass disc backed by a plug of agar and saturated potassium chloride solution. The indicator electrodes were arranged as shown in Fig. 1 (*b*).

A small remaining amount of oxygen was found in the particular batch of chromic chloride solution used. The amount in 130 ml (the cell volume) corresponded to a generation time of 6 seconds at 4·110 mA, *i.e.*, $1{\cdot}57 \times 10^{-5}$ g of oxygen per litre. This amount was determined before any titrations were performed and was used as a blank value.

Titrations were again carried out on samples saturated with air; Table II shows a comparison of the results with those found by Winkler's method.

The potential of the generator cathode against the saturated-calomel electrode was measured throughout the titrations and was found to vary between 0·85 and 0·87 volt when the generator current was 4·110 mA, *i.e.*, it was well within the supposed optimum range. From these results it was concluded that chromous ions were being prepared with 100 per cent. current efficiency.

The end-points of these titrations were obtained graphically by plotting generator-current time against indicator current, as galvanometer deflection. Typical examples of such graphs for a particular generator current are shown in Fig. 2.

Similar titrations were performed on samples prepared by James and Murray's method[12]; the results are shown in Table III.

Table II

Comparison of coulometric results with those by Winkler's method

In each titration the volume of sample used, V, was 50 ml, the generator current was 4·110 mA, the generation time for 130 ml of chromic chloride solution, T, was 6·1 seconds and the correction time, $(130-V)T/130$, was 3·8 seconds

Generation time, seconds	Oxygen found, at N.T.P.— by coulometric method, ml per litre	by Winkler's method, ml per litre	Difference, ml per litre
1073·8	5·12	5·11	+0·01
1063·4	5·05	5·06	−0·01
1080·3	5·13	5·13	0·00
1070·6	5·08	5·09	−0·01
1084·8	5·15	5·15	0·00
1068·4	5·07	5·06	+0·01

Table III

Recovery of added oxygen by the coulometric procedure

The correction time was calculated from the expression $(130-V)T/130$, in which V is the volume of sample taken (in millilitres) and T is the generation time (in seconds) for 130 ml of chromic chloride solution with a generator current of 2·080 mA

Volume of sample, ml	Amount of oxygen added, g × 10^{-5}	Generation time, seconds	"Blank" time (T), seconds	Correction time, seconds	Amount of oxygen found, g × 10^{-5}	Difference, %
With a generator current of 4·110 *mA*—						
15·0	4·458	136·4	12·3	10·9	4·456	−0·05
	4·518	138·2	12·3	10·9	4·522	+0·04
With a generator current of 2·080 *mA*—						
5·0	1·101	76·1	11·9	11·5	1·095	−0·6
	1·080	75·0	11·9	11·5	1·075	−0·5
2·0	0·4428	36·4	11·6	11·4	0·4482	+1·2
	0·4358	37·1	11·6	11·4	0·4402	+1·0

Conclusions

The results indicate that the proposed method is extremely sensitive and accurate to within less than 2 per cent. at the lowest concentration investigated, *viz.*, three parts of oxygen per hundred million parts of water. It has the additional advantage that no standard solutions and only one de-oxygenated solution are required.

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Molar Extinction Coefficients of Dithizone and Lead Dithizonate in Carbon Tetrachloride

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Methods for the direct determination of the molar extinction coefficients of dithizone and lead dithizonate are described. The optical densities of extremely pure dithizone solutions were measured at characteristic wavelengths, and the concentrations of the solutions were subsequently determined by weighing the dithizone residues after evaporation of the solvent. The molar extinction coefficient of lead dithizonate was determined on solutions of pure lead dithizonate, the lead contents of which had been determined polarographically, succinic acid being used as supporting electrolyte.

THE major difficulty encountered in attempts to determine the molar extinction coefficient of dithizone is the preparation of pure dithizone solutions having known concentrations. It is almost impossible to prepare such a solution simply by dissolving a weighed amount of the solute, since even the purest dithizone crystals invariably contain small amounts of different oxidation products.[1,2,3,4]

Attempts to determine the extinction coefficient of dithizone in carbon tetrachloride have been made by Liebhafsky and Winslow,[5] by Clifford[1] and by Geiger and Sandell,[6] but these workers describe neither the preparation of the solution nor the details of measurement. An indirect method for determining the molar extinction coefficient of dithizone has been described by Cooper and Sullivan.[2] This consists in preparing a solution of the dithizonate of lead, zinc, mercury or silver, destroying the complex, measuring the optical density of the liberated dithizone and subsequently determining the amount of metal originally bound to dithizone by a colorimetric dithizone method. The main objection is that the stoicheiometry of the reaction, which is generally obtained from changes in optical density, is assumed; it is also assumed that all the reactions go to completion.

As the purpose of our work was the study of some dithizone equilibria, none of the methods just mentioned was suitable.

Cooper and Sullivan computed the molar extinction coefficients of lead dithizonate and dithizone from the same set of results. Apart from the objection already referred to, their value for lead dithizonate was measured at only one wavelength (520 mμ) and is therefore insufficient for differential (difference) spectrophotometry.

We therefore decided to design a simple and direct method for measuring the extinction coefficient of dithizone. The method consists in preparing a solution of dithizone in carbon tetrachloride, purifying it by repeated extraction from an aqueous phase and measuring its optical density; the concentration of the solution is subsequently determined by weighing the residue after the solvent has been removed by evaporation. Once the optical density has been measured, the possible formation of oxidation products during and after evaporation of the solvent has practically no effect on the final calculated concentration of the solution, since the molecular weights of dithizone and its oxidation products differ by not more than 1 per cent.

The extinction coefficient of lead dithizonate was determined by preparing a solution of pure lead dithizonate containing no excess of dithizone and measuring its lead content by an independent polarographic method. Succinic acid was used as the supporting electrolyte.

Method

Apparatus—

Pyrex glassware was used throughout. Separating funnels were pear-shaped and made from dark "low-actinic" glass. Glassware was cleaned by soaking in chromic - sulphuric acid mixture and then rinsing with warm tap-water and finally with distilled water that had been redistilled from a Pyrex-glass still. The washing procedure was then repeated with warm 10 per cent. sodium hydroxide solution, distilled water, warm diluted nitric acid (1 + 2) and redistilled water (in that order).

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A Beckman model M pH meter was used for pH measurement.

Optical densities were measured with a Beckman DU spectrophotometer; 1-cm fused-silica cells were used and optical corrections were applied. All measurements were made at constant spectral-band width (3 mμ) and constant temperature (25° ± 0·1° C).

Organic solvent was evaporated in the apparatus shown in Fig. 1 (p. 42).

REAGENTS—

Water used in the preparation of aqueous solutions was redistilled from a Pyrex-glass still.

Carbon tetrachloride—Phosgene and other impurities were removed by distilling analytical-reagent grade carbon tetrachloride on a water bath in presence of 20 ml of a 20 per cent. sodium hydroxide solution per litre of carbon tetrachloride and a few crystals of sodium thiosulphate. This procedure was repeated three times and the final distillate was dried with anhydrous sodium sulphate. After filtration, the solvent was distilled *in vacuo* at 32° to 34° C. During purification, the carbon tetrachloride was screened from direct daylight. The addition of ethanol as preservative[7] was not necessary.

Ammonium hydroxide solution, 2 per cent. v/v—Prepared from analytical-reagent grade concentrated ammonium hydroxide that has been redistilled.

Sulphurous acid, 6 per cent.

Concentrated nitric acid—Analytical-reagent grade acid that has been redistilled.

Hydrogen peroxide, 100-volume—Analytical-reagent grade.

Succinic acid, 0·5 N.

Standard lead solution—Analytical-reagent grade lead was washed with diluted acetic acid (1 + 1), redistilled water and acetone and was then dried. A 1·0000-g portion was dissolved in 5 ml of redistilled analytical-reagent grade concentrated nitric acid and the solution was diluted to 1 litre with redistilled water. A 25-ml portion of this solution was diluted to 1 litre to give a solution containing 25 μg of lead per ml. To a 16-ml portion of this solution were added 2 ml of 0·5 *N* citric acid and 38 ml of 0·5 *N* ammonium hydroxide, and the solution was diluted to 100 ml with redistilled water. This solution contained 4 μg of lead per ml.

PROCEDURE FOR DETERMINING MOLAR EXTINCTION COEFFICIENT OF DITHIZONE—

About 0·5 g of analytical-reagent grade dithizone was dissolved in 300 ml of carbon tetrachloride, and the solution was filtered through a sintered-glass filter. The solution was then transferred to a separating funnel containing 500 ml of 2 per cent. v/v ammonium hydroxide solution, which, like all other solutions used, had been previously saturated with nitrogen. The nitrogen, which was also used to replace air in all flasks, separating funnels, etc., was freed from oxygen by bubbling it through a saturated solution of pyrogallol in 25 per cent. w/v sodium hydroxide solution. All the subsequent operations were carried out in a dark room illuminated only by red light. The solutions were thoroughly mixed by vigorous shaking, thereby facilitating the transfer of dithizone, as ammonium dithizonate, from the organic layer to the aqueous layer. By this procedure, dithizone was separated from all impurities, including its oxidation products, which all remained in the organic layer.[3,4] The aqueous layer was then separated from the organic layer and extracted several times with small amounts of carbon tetrachloride, extraction being continued until the last portion of the solvent remained colourless. The aqueous layer was then acidified with 6 per cent. sulphurous acid to convert the ammonium dithizonate to dithizone, and the finely dispersed precipitate of dithizone obtained was extracted with small portions of carbon tetrachloride. Sulphurous acid was preferred for the precipitation of dithizone because of its reducing properties. Since, according to Barnes,[8] sulphurous acid can reduce diphenylthiocarbodiazone if it has been formed, it is improbable that any oxidation could take place in presence of this acid. The purified solution of dithizone in carbon tetrachloride was separated from the aqueous layer and was further purified by treatment with 2 per cent. v/v ammonium hydroxide solution in the way described above. The purification procedure was frequently checked by computing the ratio of the optical densities at 620 and 450 mμ. At this stage, the volume of dithizone solution was 300 ml.

The dithizone solution was repeatedly washed with water until it was neutral and was then spun in a centrifuge for 15 minutes at 3000 r.p.m. and 25-cm radius to remove dispersed water droplets. The dithizone solution so obtained was saturated at slightly above room

temperature; to prevent possible precipitation during cooling, it was diluted with a small amount of nitrogen-saturated carbon tetrachloride. Optical-density measurements were made on some of this solution, after suitable dilution; the rest was used in the determination of its concentration.

The concentration was determined by weighing the residue after the solvent had been removed by evaporation in the apparatus shown in Fig. 1. By using a pipette having a capillary tip, 25·00 ml of solution were placed in flask F, and the air-stream inlet (capillary tube D) was positioned above the surface of the solution. A uniform and smooth evaporation, without loss of dithizone, could be obtained by adjustment of the air-stream velocity (by means of a screw-clip at E), the pressure and the temperature (by means of electric heater C). The temperature was never allowed to exceed 50° C.[9] In order to obtain sufficient solid for accurate weighing, a total of 300 ml of solution was evaporated in each determination. When the solvent had been removed, the residue was dried *in vacuo*. Before it was weighed, flask F was transferred to a desiccator filled with dry silica gel. The drying procedure was repeated until the residue attained constant weight.

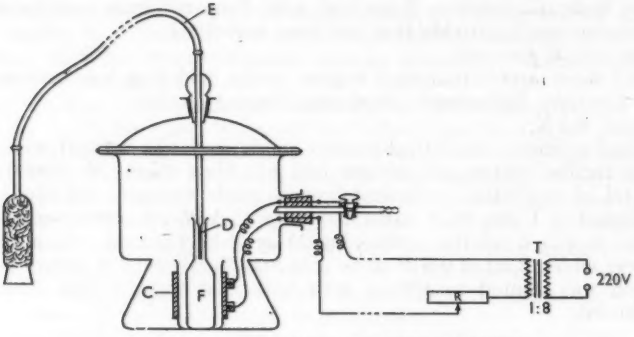

Fig. 1. Apparatus for evaporation of solvent

PROCEDURE FOR DETERMINING MOLAR EXTINCTION COEFFICENT OF LEAD DITHIZONATE—

A 100-ml portion of an approximately $21{\cdot}8 \times 10^{-6}$ *M* solution of dithizone in carbon tetrachloride was placed, by pipette, in a 250-ml separating funnel containing 40 ml of standard lead solution. At these concentrations, a solution of lead dithizonate free from excess of dithizone was obtained in the organic layer[10] after vigorous shaking. The organic layer was then transferred to a 100-ml calibrated flask, and diluted to the mark with carbon tetrachloride, if necessary. The optical density of this solution was measured at 520 and 620 mμ, the absorption maxima of lead dithizonate and dithizone, respectively.

The concentration of lead dithizonate in the solution was determined as follows. A precisely known volume (40 ml) of lead dithizonate solution was placed, by pipette, in a round-bottomed flask, and the solvent was evaporated by heating on a water bath. A 5-ml portion of concentrated nitric acid was added, and the contents of the flask were evaporated to dryness. This procedure was repeated three times and then 10 ml of concentrated hydrogen peroxide were added to ensure the complete destruction of any organic material. The contents of the flask, pure lead nitrate, were dissolved in 0·5 *N* succinic acid and quantitatively transferred to a 10-ml calibrated flask, 2 ml portions of succinic acid solution being added at a time. The lead content of this solution was then determined polarographically.[11] A similar series of experiments was carried out, starting with 25 ml of standard lead solution and 100 ml of $13{\cdot}6 \times 10^{-6}$ *M* dithizone.

DESIGN OF THE EXPERIMENT

To minimise the experimental error, the concentrations of lead and dithizone solutions were chosen so that the optical densities of the resulting lead dithizonate solutions were between 0·20 and 0·45, in which range measurements with a Beckman spectrophotometer have their greatest precision.[12] As the optical density of a solution is directly proportional to the concentration of the solute ($D = \epsilon c$), for unit pathlength the variance of the molar

extinction coefficient, V_ϵ, calculated from N pairs of values of D and c is given by the expression—

$$V_\epsilon = \frac{V_D}{\Sigma (c - \bar{c})^2}$$

in which V_D is the variance of the optical density and $\bar{c}$ is the mean concentration of the solute. It is obvious, therefore, that V_ϵ will be a minimum when $\Sigma (c - \bar{c})^2$ is made as large as possible. This can be achieved by making optical-density measurements at two values of concentration, c, as far apart as is consistent with the requirement that the optical density should be between 0·20 and 0·45. Consequently, we carried out only two series of experiments, one series at a concentration giving optical densities of about 0·200 and the other optical densities of about 0·440. In this instance, for N pairs of measurements of concentration and optical density, the variance of the molar extinction coefficient is $3 (N - 1)/(N + 1)$ times smaller than it is for equally spaced concentrations in the same range.[13]

Results

Molar extinction coefficient of dithizone—

Independent measurements of the concentrations and optical densities of twelve separately prepared dithizone solutions were made. The optical densities were measured at the primary and secondary absorption maxima and the absorption minimum of dithizone, 620, 450 and 515 mμ, respectively, and at the absorption maximum of lead dithizonate, 520 mμ; the results are shown in Table I. The values calculated for the molar extinction coefficient by computing the linear regression of optical density on concentration are shown in Table II, together with the corresponding measures of experimental error.

Table I

Optical densities of dithizone solutions

Solution No.	Concentration of dithizone, mole per litre × 10^5	Optical density at—			
		620 mμ	520 mμ	515 mμ	450 mμ
1	1·418	0·518	0·085	0·080	0·303
2	1·257	0·459	0·074	0·071	0·272
3	1·156	0·423	0·066	0·062	0·249
4	1·384	0·502	0·077	0·072	0·292
5	1·734	0·624	0·098	0·091	0·367
6	1·348	0·488	0·079	0·073	0·289
7	1·467	0·538	0·086	0·080	0·317
8	1·050	0·382	0·062	0·057	0·224
9	1·309	0·473	0·073	0·068	0·278
10	1·490	0·546	0·083	0·077	0·322
11	1·321	0·482	0·076	0·070	0·285
12	1·409	0·511	0·085	0·080	0·300

Table II

Values of molar extinction coefficient of dithizone

Wavelength of optical-density measurement, mμ	Molar extinction coefficient (× 10^{-3})	Standard deviation (× 10^{-3})	Coefficient of variation, %	95 per cent. confidence limits (× 10^{-3})
620	36·4	0·1	0·3	36·2 to 36·6
520	5·8	0·05	0·9	5·7 to 5·9
515	5·4	0·05	0·9	5·3 to 5·5
450	21·4	0·2	1·0	21·0 to 21·8

The value of the molar extinction coefficient at 520 mμ is useful for the calculations involved in differential spectrophotometry of a mixture of dithizone and lead dithizonate.

Molar extinction coefficient of lead dithizonate—

The measurements of concentration and optical density used in computing the molar extinction coefficient of lead dithizonate are shown in Table III; values of the molar extinction coefficient at 520 and 620 mμ and the measures of experimental error are shown in Table IV.

Discussion of results

Table V shows a comparison of our results with those of other workers for the molar extinction coefficient of dithizone. The large differences between our results and those of Liebhafsky and Winslow,[5] Clifford[1] and Geiger and Sandell[6] may be partly explained by the presence of impurities in the dithizone solutions used by these workers. The presence

Table III

Optical densities of lead dithizonate solutions

Solution No.	Concentration of lead dithizonate, mole per litre × 10^6	Optical density at— 520 mμ	620 mμ
1	2·789	0·204	0·008
2	2·761	0·203	0·007
3	2·838	0·208	0·008
4	2·780	0·202	0·005
5	2·741	0·202	0·006
6	2·847	0·207	0·009
7	2·847	0·209	0·008
8	2·799	0·206	0·007
9	6·020	0·441	0·021
10	5·960	0·436	0·018
11	6·008	0·438	0·017
12	5·960	0·439	0·022
13	6·020	0·437	0·019
14	6·105	0·447	0·021
15	6·057	0·441	0·022
16	6·008	0·439	0·017

Table IV

Values of molar extinction coefficient of lead dithizonate

Wavelength of optical-density measurement, mμ	Molar extinction coefficient (× 10^{-3})	Standard deviation (× 10^{-3})	Coefficient of variation, %	95 per cent. confidence limits (× 10^{-3})
520	72·9	0·3	0·4	72·4 to 73·4
620	3·9	0·3	7·8	3·3 to 4·5

Table V

Comparison of values for molar extinction coefficent of dithizone

Molar extinction coefficient at— 620 mμ	520 mμ	514 mμ	450 mμ	Ratio of molar extinction coefficients at 620 and 450 mμ	Reference
30,400	—	4740	19,000	1·60	Liebhafsky, H. A., *et al.*[5]
31,100	—	4510	18,700	1·65	Clifford, P. A.[1]
29,700	—	5400	17,800	1·61	Clifford, P. A.[1]
34,600	—	—	20,300	1·70	Cooper, S. S., *et al.*[3]
33,800	—	—	20,100	1·68	Bell, C. F.*
31,000	5700	—	19,800	1·57	Geiger, R. W., *et al.*[6]
36,400	5800	5400	21,400	1·70	This paper

* Unpublished D.Phil. Thesis, Oxford University, 1952.

of impurities, mainly oxidation products, such as diphenylthiocarbodiazone, is revealed by the low values of the ratio of the molar extinction coefficients at 620 and 450 mμ, as the oxidation products have only one absorption maximum, at 410 mμ.[14,15,16] The presence of oxidation products therefore tends to decrease the absorption maximum at 620 mμ and to increase the optical density at 450 mμ, the net result being a decrease in the ratio of molar extinction coefficients at these wavelengths. Geiger and Sandell's measurements give a ratio of 1·57; Liebhafsky and Winslow's and Clifford's ratios are slightly higher, 1·60 and 1·66,

respectively, but are significantly lower than the value computed from our measurements, $1{\cdot}70 \pm 0{\cdot}03$. The molar extinction coefficient found by Cooper and Sullivan is much closer to our value; the difference is about 5 per cent. and probably arises from an error caused by the presence of metal dithizonates in the dithizone solution, and, to some extent, to their rather complicated experimental technique, which involved the attainment of several dithizone equilibria.

Although minimum absorption of dithizone in carbon tetrachloride occurs at 515 mμ, special attention has been paid not to this wavelength but to that of 520 mμ, at which the requirements for maximum accuracy in differential spectrophotometry are better fulfilled. Calculations for both dithizone and lead dithizonate have therefore been made at 520 and 620 mμ.

By suitably designing the experiments for determining the molar extinction coefficient of lead dithizonate, it was possible to obtain fairly precise results and at the same time to reduce considerably the number of these rather tedious and complicated experiments. The precision attained in sixteen independent experiments equally grouped around two well spaced concentrations, $2{\cdot}6$ and $6{\cdot}0 \times 10^{-6}$ mole per litre, is equal to that attainable in forty-two experiments, if these experiments were equally spaced throughout the same range. The standard errors of the molar extinction coefficients at 520 and 620 mμ are approximately equal, but, in terms of the coefficient of variation (relative standard error), they amount to $0{\cdot}34$ and $6{\cdot}82$ per cent., respectively. The reduced precision at 620 mμ is due to the low optical-density values at this wavelength and to the contribution from extremely small amounts of free dithizone, which may adventitiously be present even when the solutions of lead dithizonate have been prepared in the way described.

The only previously reported value[2] for the molar extinction coefficient of lead dithizonate in carbon tetrachloride is $(68{\cdot}6 \pm 1{\cdot}9) \times 10^3$. This value is considerably lower than our value and the precision is also much less, for reasons outlined in the introductory remarks to this paper and elaborated when discussing the molar extinction coefficient of dithizone.

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The Extraction Constant of Lead Dithizonate

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The theoretical expression for the extraction constant defining partition equilibrium between metal in the aqueous phase and metal complex in the organic phase has been tested experimentally and found to be valid over a wide range of pH. The extraction of lead as its dithizonate complex into carbon tetrachloride has been investigated in the pH range 5 to 11 by using three aqueous buffer solutions containing ammonia and citrates or cyanides, the ionic strength being kept constant at 0·3. The extraction constant of lead dithizonate is $1{\cdot}4 \times 10^{-3}$ in presence of citrates and $2{\cdot}9 \times 10^{-4}$ in presence of cyanides. The reaction between dithizone in carbon tetrachloride and lead ions is extremely rapid, and the rate at which partition equilibrium is approached requires no special attention.

WHEN a weakly acidic chelating agent, HR, reacts with an n-valent cation, M^{n+}, to form a metal chelate, MR_n, according to the over-all reversible reaction represented by the equation—

$$n(HR)_o + (M^{n+})_w \rightleftharpoons (MR_n)_o + n(H^+)_w \quad .. \quad .. \quad .. \quad (1)$$

it has been shown[1,2] that the partition equilibrium for this reaction can be represented by the following expression for the extraction constant (K)—

$$K = \frac{[MR_n]_o[H^+]_w^n}{[M^{n+}]_w[HR]_o^n} \quad .. \quad .. \quad .. \quad .. \quad .. \quad .. \quad (2)$$

This expression is only a useful approximation obtained by making several assumptions,[2,3] but it is sufficiently accurate for all practical purposes. When hydrolysis of the metal ion in the aqueous phase has to be taken into account, the expression has been modified[1,4] to become—

$$K = \frac{[MR_n]_o\{[H^+]_w^n + K_{h1}[H^+]_w^{n-1} + K_{h2}[H^+]_w^{n-2} + \ldots\}}{[M]_w[HR]_o^n} \quad .. \quad .. \quad .. \quad (3)$$

in which K_{h1} and K_{h2} are the appropriate hydrolysis constants and $[M]_w$ is the concentration of all forms of metal remaining in the aqueous phase. When rearranged and applied to the lead - dithizone system, equation (3) becomes—

$$E = \frac{[PbDz_2]_o}{[Pb]_w} = \frac{K[HDz]_o^2}{[H^+]^2 + K_{h1}[H^+]_w + K_{h2}} \quad .. \quad .. \quad .. \quad .. \quad (4)$$

in which E is the extractability, *i.e.*, the ratio of the concentration of metal extracted as complex into the organic phase to that remaining in the aqueous phase. The latter concentration is defined by the expression—

$$[Pb]_w = [Pb^{2+}]_w + [Pb(OH)^+]_w + [Pb(OH)_2]_w$$

and the hydrolysis constants are defined by—

$$K_{h1} = \frac{[Pb(OH)^+]\,[H^+]}{[Pb^{2+}]} \quad .. \quad .. \quad .. \quad .. \quad .. \quad (5)$$

and

$$K_{h2} = \frac{[Pb(OH)_2]\,[H^+]^2}{[Pb^{2+}]} \quad .. \quad .. \quad .. \quad .. \quad .. \quad (6)$$

Equation (2) was first experimentally tested in a preliminary way for the extraction of zinc by dithizone[1] and later for some extractions by cupferron.[5] Thereafter, papers dealing with different metal - dithizone systems have been published,[6 to 11] but neither the general equation (3) nor its rearranged form (4), as applied to the lead - dithizone system, has been verified experimentally. The results obtained when testing this equation are the main subject of this paper.

With known hydrolysis constants, see equations (5) and (6), the determination of the extraction constant of lead dithizonate reduces to the determination of the concentrations

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of lead dithizonate and free dithizone in the organic phase in equilibrium with an aqueous phase having a known pH. The determination of these concentrations is the chief experimental difficulty.

It seemed that the problem could be solved by differential (difference) spectrophotometry, which permits the concentrations of both components in the organic phase to be determined precisely and does not in any way interfere with the previously established equilibrium. The total concentration of lead present in any form in the aqueous phase, $[Pb]_w$, can be computed from the difference between the initial concentration of total lead and the amount found in the organic phase at equilibrium.

For solving the well known equations involved in differential spectrophotometry,[12] use has been made of the molar extinction coefficients of dithizone and lead dithizonate already found.[13] It was established that solutions of dithizone, lead dithizonate and mixtures of these two compounds obeyed Beer's law in the relevant concentration range at appropriate wavelengths (520 and 620 mμ). Equal volumes of organic and aqueous phases were used throughout this work to simplify the calculations. No corrections for changes in volume were necessary, as organic and aqueous phases were pre-saturated with water and carbon tetrachloride, respectively.

If x and y are defined as follows—

$$x = \frac{[HDz]_o^2}{[H^+]_w^2 + K_{h1}[H^+]_w + K_{h2}} \quad .. \quad .. \quad .. \quad (7)$$

$$y = \frac{[PbDz_2]_o}{[Pb]_w} \quad .. \quad .. \quad .. \quad .. \quad .. \quad (8)$$

equation (4) predicts that a graph of x against y should be a straight line, the slope of which is numerically equal to K. The regression coefficient obtained by linear regression analysis of the pairs of values for x and y will be, according to the method of least squares, the best estimate of the extraction constant.

The extraction constants were determined by using aqueous phases containing citrates or cyanides in the pH range 5 to 11, as such solutions are known to be the most suitable for determining trace amounts of lead in the presence of several heavy metals, especially those encountered in biological samples.

From values of the first and second dissociation constants of lead hydroxide,[14,15] the hydrolysis constants defined by equations (5) and (6) were calculated to be $3{\cdot}33 \times 10^{-7}$ and $3{\cdot}57 \times 10^{-18}$, respectively. In view of the trace amounts involved and the constant ionic strength of the aqueous phase, the use of concentrations rather than activities throughout this work appeared to be justified.

Experimental

All glassware and carbon tetrachloride and other volatile chemicals were freed from trace metals in the usual way. In order to avoid the presence of uncontrolled amounts of foreign substances, especially dithizonate ions, which could have entered the reagents during purification procedures involving dithizone, the buffer solutions and other reagents were prepared by recrystallising the purest chemicals obtainable and then dissolving them in water. Control solutions were concurrently treated in the same way as the solutions for partition studies, and their lead contents were determined separately in each experiment. These blank values were added to the initial lead concentrations, thereby making known the total lead concentrations.

Aqueous phases having pH values between 5 and 11 were prepared by varying the ratio of citric acid to ammonium hydroxide, or citric acid to potassium cyanide, the total ionic strength being kept constant at 0·3. Three series of experiments were carried out; in the first series, the aqueous phase consisted of solutions of citric acid and ammonium hydroxide; in the second series, it consisted of solutions of citric acid and potassium cyanide; in the third series it consisted of solutions of citric acid and ammonium hydroxide having half the concentrations used in the first series, the ionic strength being maintained by adding the necessary concentration of an inert salt (sodium nitrate). The preparation of one of the aqueous solutions used in the first series of experiments is described on p. 48.

The dithizone solutions were prepared by suitably diluting concentrated solutions of twice-purified dithizone[13] with carbon tetrachloride. Dithizone solutions having three

different concentrations were used in each series of experiments. The optical densities of these solutions at 620 mμ were 0·540, 0·405 and 0·220, corresponding to $14{\cdot}83 \times 10^{-6}$, $11{\cdot}13 \times 10^{-6}$ and $6{\cdot}04 \times 10^{-6}$ mole per litre, respectively. The lead concentration in the aqueous phase always amounted to a little more than $4{\cdot}83 \times 10^{-6}$ mole per litre, and, as mentioned before, the volumes of organic and aqueous phases were the same. In the first and second series of experiments, therefore, the excesses of dithizone over the amount required for the formula $PbDz_2$ were 1·5 to 1 and 1·15 to 1, respectively; in the third series there was a 40 per cent. deficiency of dithizone.

Method

Apparatus—

All pH measurements were made with a Beckman model M pH meter fitted with a No. 4990–80 glass electrode (pH range 1 to 11, temperature range 0° to 100° C). The pH meter was calibrated at pH 4·00 and 7·00 by using standard buffer solutions obtained from the Gamma Instruments Co., Philadelphia, U.S.A. Temperature correction was not applied, as both standard buffers and solutions being analysed were kept at 20° ± 0·5° C during measurement.

Reagents—

In addition to those described previously,[13] the reagents listed below were used—

Citric acid, 0·5 N—Dissolve exactly 35·027 g of citric acid, $C_6H_8O_7.H_2O$, in 1 litre of redistilled water.

Ammonium hydroxide, 0·5 N—Dilute 35·4 ml of redistilled ammonia solution, sp.gr. 0·902, to 1 litre with redistilled water.

Potassium cyanide, 0·5 N.

Sodium nitrate, 0·5 N.

Standard lead solution—Dilute 25 ml of the lead solution containing 100 μg of lead per ml[13] to 1 litre with redistilled water in a calibrated flask. The resulting solution contains 25 μg of lead per ml ($120{\cdot}7 \times 10^{-6}$ mole per litre).

Preparation of an aqueous solution for the first series of experiments—

A 19-ml portion of 0·5 *N* citric acid, 21·0 ml of 0·5 *N* ammonium hydroxide and 4·00 ml of standard lead solution were placed in a 100-ml calibrated flask and diluted to the mark with redistilled water. The lead concentration of this and all other similarly prepared solutions was 1 μg per ml, *i.e.*, $4{\cdot}83 \times 10^{-6}$ mole of added lead per litre. As 25 ml of the aqueous phase were used in each partition experiment, the total lead content was always 25 μg *plus* the amount of lead in the blank solution, which was prepared in the way just described, but without the addition of standard lead solution.

Procedure for determining dithizone and lead dithizonate concentrations—

Dithizone was dissolved in carbon tetrachloride previously saturated with water, and a 25-ml portion of such a solution, having a known initial optical density, was equilibrated in a pear-shaped 125-ml separating funnel made from "low-actinic" glass with 25 ml of the aqueous solution described above; the aqueous solution was pre-saturated with pure carbon tetrachloride. The contents of the separating funnel were shaken for 3 minutes, and, after separation had occurred, a 1-cm optical cell was filled with the organic layer. When necessary, the separation of the layers and the removal of water droplets, which cause erroneous optical-density readings, were achieved by spinning the separating funnel and its contents, in a special trunnion-carrier, in a centrifuge at 1500 r.p.m. and 25-cm radius.

Each optical cell was filled three times with the organic layer, and optical-density readings were made at 620 and 520 mμ with a Beckman spectrophotometer, carbon tetrachloride being used as reference solution. Corrections were applied for differences between the absorptions of the two cells.

The hydrogen ion concentration at equilibrium was ascertained by transferring the aqueous layer to a beaker after the equilibration and measuring its pH.

By substituting previously obtained values for extinction coefficients[13] and solving the equations[12] for two light-absorbing components, the concentrations of lead dithizonate and free dithizone were calculated.

Results

The variation in optical density with pH for the first series of experiments, in which the aqueous phase was buffered with citric acid and ammonium hydroxide, is shown in Table I. For each pH, the corresponding pair of values for x and y were calculated from equations (7) and (8) and the regression of y upon x was estimated in the usual way[16]; the analysis of variance is shown in Table II.

Table I

Variation in optical density of organic layer with pH

Each result is the mean of three independent experiments. The initial optical densities of solutions A, B and C at 620 mμ were 0·540, 0·405 and 0·220

pH of aqueous layer	Optical density of organic layer A at—		Optical density of organic layer B at—		Optical density of organic layer C at—	
	520 mμ	620 mμ	520 mμ	620 mμ	520 mμ	620 mμ
5·12	0·090	0·542	0·069	0·401	0·045	0·220
6·30	0·174	0·445	0·137	0·346	0·066	0·191
7·21	0·339	0·239	0·324	0·186	0·164	0·106
7·95	0·371	0·181	0·325	0·096	0·221	0·048
8·52	0·362	0·131	0·334	0·064	0·205	0·026
8·57	—	—	0·338	0·058	—	—
9·20	0·338	0·059	0·328	0·031	0·206	0·022
9·90	0·294	0·027	0·264	0·022	0·184	0·014
10·33	0·295	0·026	0·246	0·019	0·181	0·006
10·88	0·174	0·011	—	—	0·100	0·004

Table II

Analysis of variance of regression

Source of variation	Degrees of freedom	Sum of squares	Variance
Regression	1	179·88	179·88
About the regression line ..	25	59·05	2·36
Total	26	238·93	

In order to assess the significance of the regression, the ratio of the variances due to regression and about the regression line was calculated. This ratio is 179·88/2·36, *i.e.*, 76·15, and for 1 and 25 degrees of freedom the 5 and 1 per cent. values of F are 4·26 and 7·28, respectively. The regression is therefore highly significant.

The regression coefficient, which is numerically equal to the extraction constant, K, was calculated to be $1{\cdot}42 \times 10^{-3}$, its standard error, s_K, being $0{\cdot}13 \times 10^{-3}$. The third series of experiments, in which a buffer solution having the same composition but only half the concentration was used, gave the value $(1{\cdot}39 \pm 0{\cdot}18) \times 10^{-3}$. This value is not significantly different from the value in the more concentrated solution. Similarly, the extraction constant for the aqueous phase containing potassium cyanide and citric acid was found to be $(2{\cdot}93 \pm 0{\cdot}63) \times 10^{-4}$.

All the results obtained are shown in Table III. The difference between the extraction constant obtained in the second series and that in the first or third series is obvious and amounts to 1 logarithmic unit.

Table III

Extraction constants and their measures of variation

Series	Composition of aqueous phase	Value of K	Value of s_K	Value of s^2
First	Citrates	$1{\cdot}42 \times 10^{-3}$	$0{\cdot}13 \times 10^{-3}$	2·362
Second	Cyanides	$2{\cdot}93 \times 10^{-4}$	$0{\cdot}63 \times 10^{-4}$	13·248
Third	Citrates and sodium nitrate	$1{\cdot}39 \times 10^{-3}$	$0{\cdot}18 \times 10^{-3}$	5·963

The rate of the reaction between dithizone and lead ions was also investigated at four different pH values covering the whole range of interest, namely, 4·31, 6·24, 7·48 and 9·88. The samples were shaken for periods from 1 to 1200 seconds, *i.e.*, from 3 to approximately

3600 shakes. The reaction proceeded so rapidly that no kinetic parameters could be calculated, and the variation in optical density with time showed no trend. The results for the ammonium hydroxide - citric acid buffered phase (pH 7·48), for which variations between single measurements are within the limits of experimental error, were—

Time of shaking, seconds ..	1·5	3·0	4·5	6	9	30	60	180	300	600	1200
Optical density at 520 mμ ..	0·347	0·354	0·351	0·351	0·350	0·354	0·354	0·364	0·353	0·356	0·353

Discussion of results

The analysis of variance shows that the relationship between the variables x and y, as defined by equations (7) and (8), is linear, *i.e.*, the extraction constant of lead dithizonate is virtually independent of the pH of the aqueous phase. The ratio of the concentrations of lead in the organic and aqueous phases is also independent of the amount of free dithizone in the organic phase after equilibrium has been attained. Equation (4) therefore represents the equilibrium for the lead - dithizone system in carbon tetrachloride extremely well over the pH range 5 to 11. On the other hand, the application of equation (2) to the same system does not appear to be justified, at least not over the pH range in which lead ions are hydrolysed. The linearity also gives further evidence that one bivalent lead ion combines with two dithizone molecules. This result is compatible with those found by direct analysis,[17] a method involving continuous variations in a two-phase system,[18] a radiochemical method,[19] reversion[20] and methods involving continuous variations and continuous addition in a monophase consisting of 20 per cent. of carbon tetrachloride, 8 per cent. of water and 72 per cent. of methanol and in a two-phase system of carbon tetrachloride and water.[21] However, it is in marked disagreement with Liebhafsky and Winslow's result[22]; they ascribe the ratio of lead to dithizone as 1 to 1.

The results in Table III show that the numerical value for the extraction constant depends on the composition of the aqueous phase. In the presence of cyanides, the extraction constant is ten times smaller than in the presence of citrates. This means that if all other factors, *e.g.*, pH and excess of dithizone, are constant, the amount of lead extracted will be smaller when the aqueous phase contains cyanides than when it contains citrates. This is in accordance with Sandell's qualitative description[23] and with observations by other workers,[24,25] who have reported differences in the amounts of lead extracted in the presence of different anions in aqueous phases having the same pH.

The statistically insignificant difference between the extraction constants obtained in the first and third series of experiments indicates that the value of the extraction constant does not change if the qualitative composition remains the same and the ionic strength is maintained by adding a neutral electrolyte.

The extractability of lead as lead dithizonate was previously studied quantitatively by Babko and Pilipenko[11] and Koroleff.[9] Both papers refer to the narrow pH range around 4, which has no practical value for analytical purposes. Babko and Pilipenko expressed their results as instability constants, but did not describe the composition of the aqueous phase by which a definite pH value had been achieved. Statistical treatment of their results shows that the relative standard error of their instability constants amounts to more than 41 per cent. A comparison of their values and ours is therefore difficult. Koroleff made only one measurement for each aqueous buffer, but his results for similar systems have a similar order of magnitude.

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A Differential Method of Photometric Analysis

Part I. Application to Solutions containing One Component

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Reilley and Crawford's differential spectrophotometric method, whereby a positive deviation from Beer's law is used to produce an expansion of scale, has been applied to the analysis of one-component solutions containing the perchlorates of nickel, cobalt, copper and chromium.

Although the scale expansion used was low in relation to the capabilities of the method, the accuracy and reproducibility were of the same order as those obtained by other workers using Bastian's differential method.

DIFFERENTIAL spectrophotometric methods for determining concentrations have been advocated on theoretical grounds since 1939, and the method whereby solute at known concentration is used as a reference solution has been used since 1949 by various workers[1 to 8] in the analysis of systems containing one major constituent. The method was largely developed by Bastian and his co-workers,[1,2,3] who adjusted the concentrations of reference and test solutions so as to bring the optical-density reading into the optimum range of performance of the instrument, *i.e.*, between 0·2 and 0·6. Bastian claimed a thirteen-fold increase in sensitivity by this procedure, but the method has the disadvantage of not being applicable to reference solutions having an optical density greater than 1·74. This limitation is due to the wide slit required to obtain the full balance with the reference solution in the beam, which causes non-monochromatic radiation to pass through the solution. In these circumstances there is a negative deviation from Beer's law and a consequent decrease in the attainable accuracy of measurement.

Hiskey[4] pointed out that a large increase in sensitivity could be obtained by arranging a positive deviation from Beer's law. A method whereby this could be achieved was proposed by Reilley and Crawford,[9] who gave as the necessary condition the abandonment of the "darkness" reference. In terms of experimental procedure the null balance with the reference solution in the beam, found by using the slit-width and sensitivity controls, was no longer carried out. Reilley and Crawford derived an expression for the gain in accuracy, which was dependent only on the concentrations of the reference solutions used. The application of the method was considered to be worthy of investigation. Investigations have also been

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made by Banks and his co-workers[10,11] in the determination of neodymium in neodymium - yttrium mixtures and uranium in uranium - niobium mixtures, the experimental procedure being substantially the same as that devised by us.

Method

No procedure was explicitly described by Reilley and Crawford, and in consequence we have devised a procedure suitable for use with a Unicam SP500 spectrophotometer.

It is assumed that the concentration of one component in a large number of samples is required, the approximate composition of the samples being known. It is necessary to prepare a calibration curve of concentration against transmittance, to which the sample readings are referred.

For a component present at a level of $c \pm x$ g per litre, it is recommended that the reference solutions for the ends of the calibration curve contain the desired component in concentrations of $(c + 2x)$ and $(c - 2x)$ g per litre. In addition, three more solutions having intermediate concentrations should be prepared, such that the range $(c + 2x)$ to $(c - 2x)$ is divided into four equal concentration intervals. If accurate volumetric apparatus is used, only the concentrations of the extreme solutions need be determined chemically.

Procedure—

Determine the end-points of the scale as described below—

(*i*) Place the standard solution (R) of lower concentration in the first reference cell and the standard solution (R′) of higher concentration in a second cell.

(*ii*) Switch to position No. 1, thus bringing the transmission scale into circuit, expose the photocell, and, with solution R in the beam and the transmission scale at 100 per cent., set the galvanometer to zero by using the slit-width control.

(*iii*) Introduce solution R′ into the beam, set the transmission scale to zero, and then set the galvanometer to zero by using the dark-current control.

(*iv*) Repeat stages (*ii*) and (*iii*) successively until the galvanometer balance is maintained both with solution R in the beam and 100 per cent. transmission and with solution R′ in the beam and zero transmission.

To determine the remaining points, introduce each of the intermediate standard solutions into the beam in turn, and balance the resulting galvanometer deflection with the transmission scale.

Plot a graph of percentage transmission against concentration. The concentration of any sample solution can then be determined by checking the ends of the transmission scale, introducing the sample solution into the beam and balancing the galvanometer deflection with the transmission scale. The concentration can be read directly from the curve.

Note that there is a limitation to the method, related to the balancing voltage attainable by adjusting the dark-current control. It was found that, with the instrument used, it was impossible to balance the transmission scale at zero with solution R′ in the beam if the transmittance of solution R′ was greater than 0·22, which corresponds to an optical density of less than 0·66. This limitation does not constitute a serious defect in the method, as differential methods in general have little or no advantage for solutions having optical densities less than 0·66.

Instrument used and systems studied

A Unicam SP500 spectrophotometer and 4-cm fused-silica absorption cells were used. The systems studied were aqueous solutions of the perchlorates of nickel^{II}, cobalt^{II}, copper^{II} and chromium^{III}. These systems were chosen because previous work by us had shown them to be suitable for analytical measurements in that they remain unchanged over long periods, being neither oxidised nor hydrolysed, and are relatively unaffected by variations in pH and temperature. In addition, they obey Beer's law, are highly soluble in water and have extinction coefficients of about 10, so that a wide range of concentrations can be investigated. The wavelengths chosen for measurement were those of absorption maxima determined on the instrument used in this investigation. This ensured maximum sensitivity. Table I shows the concentrations of reference solutions, wavelengths of measurement and calculated scale expansions for the systems investigated.

TABLE I

CALCULATED SCALE EXPANSIONS FOR VARIOUS SOLUTIONS

Metal present	Wavelength used, mμ	Concentration of solution R, g per litre	Concentration of solution R′, g per litre	Calculated scale expansion*
Nickel	393	2·4000	2·7568	15
Cobalt	510	0·7835	1·0970	8
Copper	815	0·7900	1·1850	10
Chromium	410	0·9459	2·2070	18
	580			20

* Scale expansion is given by the expression $100/(T_R - T_{R'})$, where T_R and $T_{R'}$ are the percentage transmittances of solutions R and R′, respectively.

The concentrations of the reference solutions, R and R′, were determined gravimetrically, nickel as nickel dimethylglyoxime, cobalt as cobalt anthranilate, copper as cuprous thiocyanate and chromium as barium chromate.

RESULTS

Table II shows the results derived from the means of ten sets of duplicate readings made at different times by one observer, the same instrument and cells being used throughout; the means and coefficients of variation of these sets of results are also shown.

TABLE II

GRAVIMETRICALLY AND PHOTOMETRICALLY DETERMINED CONCENTRATIONS OF VARIOUS SOLUTIONS

Metal present	Nickel	Cobalt	Copper	Chromium	
Wavelength used, mμ	393	510	815	410	580
Concentration found photometrically, g per litre	2·4081	0·9401	0·9876	1·5763	—
	2·4065	0·9417	0·9856	1·5754	—
	2·4065	0·9371	0·9837	1·5770	—
	2·4081	0·9417	0·9856	1·5794	—
	2·4115	0·9355	0·9837	1·5803	—
	2·4132	0·9425	0·9866	—	1·5778
	2·4013	0·9417	0·9866	—	1·5794
	2·4098	0·9402	0·9856	—	1·5786
	2·4115	0·9387	0·9856	—	1·5770
	2·4146	0·9395	0·9894	—	1·5778
Mean concentration found photometrically, g per litre	2·4091	0·9399	0·9860	1·5779	
Concentration found gravimetrically, g per litre	2·4115	0·9402	0·9875	1·5770	
Mean error, %	−0·10	−0·03	−0·15	+0·06	
Coefficient of variation	0·16	0·24	0·17	0·10	

DISCUSSION OF RESULTS

The mean errors and coefficients of variation shown in Table II can be qualitatively compared with those of other workers' results by differential methods. No exact comparison can be made, as the variation between instruments could well be larger than any variation due to the difference between the techniques, and, in addition, the sensitivities of the systems investigated vary widely. Nickel and copper have been studied by Bastian,[1,2] and nickel by Sutcliffe and Peake[8]; uranium has been studied by Bacon and Milner,[5] Susano, Menis and Talbot[6] and Steele,[7] all of whom used Bastian's method in principle. There is considerable variability among the statistical parameters, but it is not possible to conclude that the more sensitive uranium system has been analysed with greater accuracy than the less sensitive nickel and copper systems. A comparison of these results with those obtained when Reilley and Crawford's method was used by us and by Banks and his co-workers[10,11] shows little difference in either absolute or relative accuracy. For our results this is to be expected, as our increase in scale expansion is roughly the same as that claimed by Bastian[1] for the nickel system (thirteen-fold). Some of the errors found by various workers when Bastian's method was used are shown in Table III.

TABLE III

ERRORS IN DETERMINATION OF CONCENTRATION BY BASTIAN'S METHOD

System	Number of results	Mean error, %	Coefficient of variation	Reference
Nickel, as perchlorate ..	10	+0·02	0·06	Bastian, R.[1]
	8	−0·03	0·05	
	4	−0·05	0·06	
Copper, as perchlorate ..	8	−0·11	0·29	Bastian, R.[3]
Nickel, as perchlorate ..	19	+0·19	2·44	Sutcliffe, G. R., *et al.*[8]
	18	+0·10	4·58	
Uranium, as U_3O_8 ..	15	0·00	0·04	Bacon, A., *et al.*[5]
	7 (duplicates)	+0·33	0·11	Steele, T. W.[7]
Uranium	11	−0·15	0·75	Susano, C. D., *et al.*[6]

The number of results in Table III is not sufficiently large to warrant the attachment of any exact significance to the statistical parameters. Further, the calculation of the coefficient of variation gives a much higher value for Sutcliffe and Peake's results, as the nickel content of their test systems was never greater than 31 per cent., whereas the systems investigated by Bastian contained not less than 98 per cent. of nickel.

Banks, Spooner and O'Laughlin[10] determined neodymium, as perchlorate, in neodymium - yttrium mixtures; they used Reilley and Crawford's method[9] and obtained a mean error of +0·23 per cent. and a coefficient of variation of 0·45 per cent. for three determinations.

The results found for the uranium - niobium system by Banks, Burke, O'Laughlin and Thompson[11] cannot be used, as the true concentrations were not given. Again, the results are insufficient for exact comparison, but they are of the same magnitude as those obtained by us.

CONCLUSIONS

Although Reilley and Crawford's method has been tested under unfavourable conditions, *i.e.*, the concentrations of the reference solutions were such as to give low scale expansions, the results for the systems nickel, copper, cobalt and chromium, as perchlorate, have been shown to be as accurate and reproducible as those obtained by Bastian's method. The unfavourable nature of the conditions was due to the fact that the same concentration intervals between reference solutions were to be used in investigations of multi-component systems; these intervals are large owing to the necessity of allowing for absorption caused by interfering species.

The experimental work described in this paper contains part of a thesis submitted by one of us (S.D.R.) for the Ph.D. degree of the University of London. The same author expresses his thanks to the Central Research Fund of the University of London for a grant for the purchase of silica absorption cells used in this work.

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The Volumetric Determination of Uranium after Reduction by Lead in Dilute Perchloric Acid Solution

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A rapid and convenient volumetric method is described for determining uranium; it is suitable for high-grade ores and oxide concentrates. A dilute boiling solution of the sample in perchloric acid is reduced with granular lead and cooled under an atmosphere of carbon dioxide; after decanting the solution and adding ferric perchlorate, it is titrated with standard potassium dichromate solution. An intensive study has been made to determine the nature of the interference caused by certain elements. The elements found to interfere can be removed by precipitation with cupferron in the perchloric acid solution. Several features of this separation have been investigated. The results obtained for uranium oxide samples by the proposed method are compared with those by a standard gravimetric method.

It is fairly generally considered that the most accurate volumetric method for determining uranium involves reduction with zinc amalgam or granular lead in acid solution and then titration with ceric sulphate or potassium dichromate solutions. Both reductions are carried out in a reductor column; in routine work, when many samples have to be analysed, this reduction step is slow and tedious because each sample requires individual attention. The use of liquid amalgams is sometimes recommended, but does not result in any real saving of time.

To decrease the time taken for the reduction of a batch of samples, several methods have been proposed in which liquid reductants, such as stannous chloride[1] or titanous chloride solutions,[2,3] are used. These methods are far more rapid because many samples can be reduced simultaneously. They have, however, other disadvantages, *e.g.*, in the stannous chloride method a large excess of the reductant is undesirable and, in the absence of a suitable indicator for observing the end-point, the approximate amount of uranium must be known. In the titanous chloride method the precipitation of metallic copper indicates completion of the reduction of uranium; any delay can, as the authors state, lead to low results owing to atmospheric oxidation of uranium, which is known to be catalysed by the presence of copper. Commercial titanous reagents have proved unsatisfactory, as they give high blank values, and it is necessary to prepare the reagent from pure oxide or metal. Both these preparations are lengthy and involved. As the precipitation of metallic copper is slow there is a tendency to over-step the end-point of the reduction; if this occurs, results are high because a large excess of titanous solution increases the blank value.

Because of the difficulties associated with the use of liquid reductants, the use of metals —so convenient in many respects—was reconsidered, but under conditions permitting the reduction of many samples to be more speedily effected. Some attempts have already been made in this direction, *e.g.*, uranium has been reduced by immersing a zinc spiral in the hot solution containing hydrochloric acid[4]; reduction is rapid, but undesirable features are the partial reduction of uranium to the tervalent state and excessive consumption of reductant by the acid. The use of powdered antimony[5] has also been suggested, but this is a slow and uncertain method.

In the proposed method uranium is reduced by granular lead in boiling dilute perchloric acid solution and cooled under an atmosphere of carbon dioxide. The solution is poured into a beaker and the lead washed by decantation. Ferric perchlorate solution is added and the ferrous ions formed, equivalent to the uranium, are titrated with standard potassium dichromate solution in the presence of phosphoric acid, sodium diphenylamine sulphonate solution being used as indicator.

When this method is applied to industrial samples it is necessary to separate the elements reduced with the uranium. As these elements are precipitated by cupferron as chloroform-soluble complexes, this separation was used for the preliminary purification of uranium. The precipitation and chloroform extraction of these elements in perchloric acid solution has formed part of this investigation because, although cupferron has been extensively studied

as a precipitant in sulphuric and hydrochloric acid solutions, no information was found for perchloric acid medium.

Experimental

Granular lead was used for the reduction because it has been shown[6] that it possesses advantages over several other reductants, such as amalgamated zinc, when used in a conventional reductor column. Hence there is no reduction beyond the quadrivalent state, it is not "poisoned" by nickel and the reduction is more specific for uranium. It was considered probable that these advantages would still hold at elevated temperatures.

Perchloric acid was chosen as the medium for reducing uranium not only because reduction is rapid but also because it is an effective solvent for uranium-bearing samples, such as oxide concentrates. Further, it is the acid commonly used for the oxidation of organic matter that may be introduced in the preliminary separation of interfering elements. Sulphuric acid was unsatisfactory, as the coating of lead sulphate formed on the lead prevented

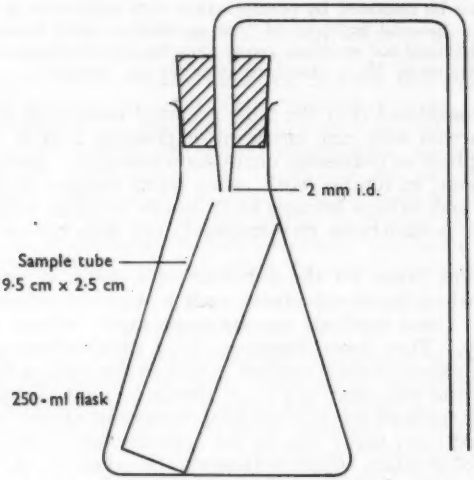

Fig. 1. Apparatus for the reduction of uranium

complete reduction. A combination of sulphuric and hydrochloric acids prevented coating of the lead, but gave blank values for the titration about three times those for perchloric acid. A small amount of hydrogen sulphide is formed when these acids are used, and this may account for the high blank value. A limited number of tests showed that reduction may be satisfactory in hydrochloric acid solution and that it should be possible to carry out the reduction with a mixture of perchloric acid (if required for oxidation of organic matter) and hydrochloric acid. However, the mixed acids do not appear to have any advantage and the large precipitate of lead chloride that separates may be undesirable.

Reduction and titration of uranium—

Two commercial sources of lead, namely, Merck's and Fisher's granular lead ("30 mesh"), have been found satisfactory. The 40-mesh particles were removed by screening. Five grams were used for the reduction, but the amount was varied from 4 to 6 g with satisfactory results. (A thimble is a sufficiently accurate measure of the amount of lead required.)

The reduction of up to 0·5 g of uranium is complete after 15 minutes' boiling; boiling for 27 minutes did not affect the results. A standard time of 20 minutes was chosen. Boiling should not be too vigorous because the acid may become too concentrated and prevent complete reduction.

For a boiling time of 20 minutes and with 5 g of lead, the perchloric acid concentration may vary from 2 to 4 M; a concentration of 3 M was used in subsequent work.

To avoid the possibility of re-oxidation of the uranium the solution was cooled under an atmosphere of carbon dioxide in the apparatus shown in Fig. 1. This is the conventional

apparatus for cooling a solution in an inert atmosphere, modified by placing a sample tube containing about 15 ml of perchloric acid in the flask to receive the sodium carbonate solution. This modification was necessary for samples containing phosphate, because, with the conventional apparatus, uranous phosphate was precipitated owing to the lowering of the acid concentration by the sodium carbonate.

No difficulty was experienced in separating the solution from the lead by decantation, as in the presence of acid the lead particles tend to cohere.

The solution was diluted to approximately 300 ml before titration, which gave a perchloric acid concentration of approximately 0·6 *M*. Perchloric acid at 0·3 to 0·9 *M* was also found to be satisfactory. The end-point of the titration with sodium diphenylamine sulphonate solution as indicator is extremely sharp. The blank value for the reduction and titration is 0·08 ml of 0·05 *N* potassium dichromate for 0·2 ml of 0·3 per cent. indicator solution.

Effect of other elements—Each element tested was added to a solution of uranium and treated as described under "Procedure," p. 60, paragraph (*d*). The solutions were titrated with 0·05 *N* potassium dichromate prepared from U.S. Bureau of Standards standard potassium dichromate. The theoretical uranium equivalent was used for calculating the result. The elements listed below were found to have no significant effect on the result (the maximum amount in milligrams of each element is given in brackets).

> Cobalt (20), nickel (20), platinum (1), bismuth (20), cerium (200), chromium (10), arsenic (45), manganese (2), mercury (10), osmium (5), cadmium (5), thallium (30), copper (10), silver (20), gold (5), selenium (5) and tin (10).

Of these elements copper, silver, gold and tin were plated on the lead. A portion of the tin is precipitated when the solution is heated to fumes with perchloric acid and remains insoluble on dilution with water. As may be expected, iron, rhenium and vanadium are reduced and react with the titrant.

The presence of molybdenum, added as either ammonium or sodium molybdate, catalyses the decomposition of the perchloric acid, and the chlorine evolved inhibits the reduction of the uranium. Not more than 0·1 mg may be present. Antimony is precipitated and co-precipitates some of the uranium, as shown by the following results—

Antimony present, mg ..	20	5	1	1
U_3O_8 added, g	0·2002	0·2001	0·2006	0·2000
U_3O_8 found, g	0·1919	0·1986	0·2005	0·2002

Hence the presence of more than about 1 mg should be avoided. Niobium interferes similarly, although the effect is less pronounced—up to 10 mg are permissible. Palladium separates on the lead in thin metallic flakes, which are readily detached from the lead and carried over into the solution during decantation. This metal has a reducing action on the uranium and causes high results; however, the effect is negligible for amounts up to 1 mg. A similar effect was observed with tellurium, and amounts larger than 1 mg should not be present. When more than 10 mg of tungsten are present, some is precipitated when the solution is heated to fumes with perchloric acid. On dilution the soluble portion is partly reduced by the lead, but appears to be rapidly re-oxidised when the solution is decanted. The effect of this element is shown by the following results—

Tungsten added, mg ..	5	10	30
U_3O_8 added, g	0·2004	0·2001	0·2007
U_3O_8 found, g	0·2008	0·2002	0·2013

Small amounts may be tolerated, but amounts larger than 20 mg should be avoided. The amount of titanium present should be less than 0·1 mg, as shown by the following results—

Titanium added, mg ..	70	5	0·5	0·15
U_3O_8 added, g	0·2005	0·2010	0·2008	0·2012
U_3O_8 found, g	0·1841	0·1923	0·1987	0·2005

It is known that titanium catalyses the atmospheric oxidation of uranium[7] and the foregoing results can be explained on this basis. The presence of nitrate or free nitric acid in small amounts causes erroneous results and with large amounts the end-point of the titration cannot be observed. These substances are, however, removed by heating to fumes with perchloric

acid. If sulphate or free sulphuric acid is present the lead becomes coated with lead sulphate and low results are obtained. The following results show the extent of the interference—

Sulphuric acid added, as SO_4^{2-}, mg ..	300	60	24
U_3O_8 added, g	0·3002	0·3002	0·3003
U_3O_8 found, g	0·2977	0·2985	0·3004

Hence not more than 30 mg of SO_4^{2-} may be present. Addition of 10 ml of hydrochloric acid prevents the interference of sulphate, but the blank value of the titration is approximately trebled. Sulphate in the uranium oxide concentrates (see Table III, p. 61) was completely removed by ignition at 950° C for 1 hour before solution of the sample. If the reduction is preceded by a cellulose-column separation of the uranium, sulphate is separated together with other interfering substances and the ignition step is then unnecessary. In the early stages of this investigation phosphate greater than 0·5 mg of PO_4^{2-} gave low results owing to precipitation of uranous phosphate when, during cooling of the uranous solution, the acidity was lowered at the point of entry of the sodium carbonate into the solution. As mentioned previously this was avoided by the use of the modified apparatus (see Fig. 1). Under these conditions up to 20 mg of phosphate, as PO_4^{2-}, may be present without disturbance.

Results

The results in Table I show the accuracy attainable by the method in the absence of interfering elements. No results have been rejected from this series.

Table I

Reduction and titration of uranium solutions

Uranium oxide prepared by solvent extraction in combination with solid absorbents[8,9] was used for preparing the pure uranium solution. The purity of this oxide has been shown by spectrographic analysis to be greater than 99·95 per cent.

Uranium added, g	Uranium found, g	Difference, mg
0·3004	0·3005	+0·1
0·3005	0·3007	+0·2
0·3006	0·3007	+0·1
0·3004	0·3009	+0·5
0·3005	0·3005	0
0·3002	0·3002	0
0·2000	0·2002	+0·2
0·2000	0·2000	0
0·1008	0·1012	+0·4
0·0507	0·0507	0
0·0502	0·0503	+0·1
0·0506	0·0508	+0·2
0·0503	0·0500	−0·3

The separation of interfering elements—

Elements that interfere in the reduction and titration of uranium can be separated by a cellulose - alumina column method.[10,11] The results by this method are shown in Table III. The separation has been extensively studied and is now a generally accepted method. A more rapid alternative method is the cupferron precipitation with extraction of the cupferrides with chloroform.

Concentration of perchloric acid for the precipitation—At a concentration less than 2·4 *M* in perchloric acid, a yellow precipitate of uranyl - cupferron complex is formed. Although the precipitate is slight at 2·4 *M*, precipitation is virtually complete at 0·3 *M*. As there is some decomposition of the cupferron precipitate above 3·6 *M* in perchloric acid the concentration was kept between 2·5 and 3 *M*.

Precipitation and extraction of interfering elements—In these tests the aqueous phase was adjusted to 100 ml and cooled to below 10° C. Ten millilitres of 6 per cent. cupferron solution were added and after the solution had been set aside for 5 minutes the precipitate was extracted once with 10 ml and then with four 5-ml portions of chloroform. The elements remaining in the aqueous phase, except niobium, were determined by photometric methods —iron with 1 : 10-phenanthroline, titanium with hydrogen peroxide, vanadium with sodium

tungstate and molybdenum with stannous chloride. Niobium was determined gravimetrically on a micro scale by precipitation with tannic acid. The results are shown in Table II.

TABLE II

AMOUNT OF ELEMENT LEFT IN THE AQUEOUS PHASE AFTER PRECIPITATION WITH CUPFERRON AND EXTRACTION WITH CHLOROFORM

Two milligrams of each element were added before precipitation

Concentration of perchloric acid present, *M*	Amount of element in aqueous phase				
	Iron, mg	Titanium, mg	Vanadium, mg	Niobium, mg	Molybdenum, mg
2·4	0·024	0·025	<0·01	<0·05	0·070
3·0	0·017	0·075	<0·01	<0·05	0·054
3·6	0·023	0·087	<0·01	<0·05	0·056

LOSS OF URANIUM IN THE EXTRACTION—

The amount of uranium extracted by chloroform was investigated as a function of (*a*) the amount of iron present, (*b*) the concentration of uranium and (*c*) the number of extractions with chloroform.

It was noticed that with some separating funnels there was a significant leakage of the aqueous phase from the stopcock, and funnels were selected to avoid errors from this source. A lubricant consisting of starch paste and glycerine was used in preference to a petroleum jelly.

(*a*) *Extraction of uranium as a function of the amount of iron precipitated by cupferron*—Although iron is not the only element precipitated by cupferron it was selected for examination because it is normally the major element present in the precipitates from oxide concentrates and also from many uranium ores.

The solution contained 0·1 g of U_3O_8 in 100 ml of 3 *N* perchloric acid and from 3 to 30 mg of iron. The iron was precipitated by adding excess of cupferron solution and was extracted with four 10-ml portions of chloroform. A photometric method[12] was used to determine uranium in the chloroform, and the results were—

Iron added, mg	3	6	30
Uranium in chloroform, mg ..	0·06	0·05	0·19

(*b*) *Extraction of uranium as a function of the concentration of uranium*—The experimental conditions were as described under (*a*), except that 0·04 to 1·0 g of U_3O_8 and 6 mg of iron were present. The results were—

Uranium added, as U_3O_8, mg ..	40	80	300	1000
Uranium in chloroform, mg ..	0·054	0·058	0·18	0·21
Uranium in chloroform, %	0·13	0·07	0·06	0·02

For the amount of uranium that would normally be present for a macrovolumetric determination (<0·3 g of U_3O_8) the percentage loss of uranium would therefore be small.

(*c*) *Extraction of uranium as a function of the number of extractions*—The solution contained 8 mg of iron and 200 mg of U_3O_8 in 100 ml of 3 *M* perchloric acid, and 10 ml of 6 per cent. cupferron solution were added to precipitate the iron. The precipitate was extracted with six 10-ml portions of chloroform, with 3-minute intervals between extractions. Uranium was determined in the individual portions of chloroform, and the results were—

Number of extraction	1	2	3	4	5	6
Uranium in chloroform, mg ..	0·098	0·010	0·005	0·010	0·011	0·006

Of the uranium extracted the major portion is found in the first extract containing the bulk of the iron precipitate. The loss of uranium in the chloroform extracts is therefore not due to any appreciable solubility of the aqueous phase, but either to the extraction of uranium co-precipitated with the iron precipitate or to physical entrainment of the aqueous phase in the chloroform saturated with the iron precipitate. The latter conclusion is supported by the observation that the first chloroform layer, which dissolves most of the precipitate, is cloudy, whereas chloroform from the subsequent extractions is crystal clear. The analytical significance of these observations is that further extractions beyond those required to remove the bulk of the precipitate will not appreciably affect the total loss of uranium.

To summarise, the amount of uranium lost in the chloroform extraction is small, and moderate variations in the amount of cupferron precipitate, in the concentration of the uranium in the aqueous phase and in the number of extractions with chloroform do not materially affect the amount of uranium lost in the extraction. This facilitates the application of corrections for the amount of uranium lost when a high degree of accuracy is required. This conclusion is supported by the results of determinations of uranium in the chloroform extracts of fourteen samples of South African uranium oxide concentrates. The over-all variation of the uranium in the extracts did not exceed 0·15 mg and the average loss was 0·04 per cent. on a 0·5-g sample.

Method

Reagents—

Ferric perchlorate solution—Add 200 ml of 70 per cent. perchloric acid to 120 g of ferric chloride, $FeCl_3.6H_2O$, and evaporate to the appearance of dense fumes of perchloric acid. Continue heating with evolution of fumes for about 10 minutes. Cool, add 50 ml of water, and again heat to fumes for about 10 minutes. Repeat the addition of water and heating to fumes once or twice more to ensure the removal of hydrochloric acid. Cool, and dilute to 1 litre.

Cupferron solution—Fresh cupferron, as indicated by its white or pale-buff colour, should be used. Dissolve 6 g of the solid in 100 ml of water.

Sodium carbonate solution—Dissolve 200 g of anhydrous sodium carbonate in water, and dilute to 1 litre.

Phosphoric acid, diluted (1 + 1)—Dilute 500 ml of analytical-reagent grade orthophosphoric acid with 500 ml of water.

Potassium dichromate solution, 0·05 N—Dissolve 2·452 g of potassium dichromate in water, and dilute to 1 litre. To standardise this solution, dissolve 0·3 g of pure U_3O_8 in 25 ml of perchloric acid, and continue as described under "Procedure," paragraph (*b*).

Granulated lead—Lead of about 30 to 40 mesh is required. Remove the —40-mesh particles by screening.

Perchloric acid, 70 per cent. w/w.

Chloroform—Analytical-reagent grade.

Sodium diphenylamine sulphonate indicator solution, 0·3 per cent.

Marble (or calcite) chips, small.

Procedure—

(*a*) Transfer 0·35 g of sample to a flat-bottomed 1-inch porcelain dish, place in a furnace at about 200° C, raise the temperature to 950° C, and keep the sample at this temperature for about 30 minutes to expel sulphate.

(*b*) Transfer the sample to a squat 500-ml beaker, add 25 ml of perchloric acid, and heat until the perchloric acid boils gently. If ignition is omitted and organic matter is present, add 5 ml of nitric acid as well as the perchloric acid. Precautions should be taken to prevent excessive loss of perchloric acid. Continue boiling the solution until the sample is decomposed and only the silica remains suspended in the solution. Wash down the walls of the flask, and again heat the solution until the perchloric acid fumes, and boil for about 30 seconds. Cool, dilute to about 50 ml with water, and boil for approximately 1 minute to expel chlorine.

(*c*) Cool the solution to below 8° C in ice - water, and transfer it to a 150-ml separating funnel by means of ice-cold water. Dilute to 100 ml with cold water. Mix the solution in the separating funnel, add 10 ml of cupferron solution, and mix. Set aside for 5 to 10 minutes, add 10 ml of ice-cold chloroform, and shake the separating funnel for about 15 seconds. Allow to settle for about 2 minutes, and run off the chloroform. Add 1 ml of cupferron solution, and note whether any further precipitation takes place. If necessary, add more cupferron solution to effect complete precipitation. Add 10 ml of chloroform, shake the funnel for about 15 seconds, and again allow the layers to separate. Run off the chloroform. Continue the extraction with chloroform until the extract is colourless. (Usually three or four extractions are required.) Discard the chloroform extracts. (See Note.)

(*d*) Transfer the aqueous solution to a squat 500-ml beaker, add 2 ml of nitric acid, evaporate to fumes, and allow the perchloric acid to boil gently for about 1 minute. Cool, wash down the sides of the beaker, evaporate to the appearance of fumes, and again boil the perchloric acid for about 1 minute. Repeat this step once more to ensure complete

expulsion of nitric acid. Reduce the volume of perchloric acid to 15 ± 3 ml. Dilute to about 40 ml, boil for 1 minute to expel chlorine, transfer to a wide-necked 250-ml conical flask, and dilute to 60 ± 5 ml.

(*e*) Add 5 ± 1 g of granulated lead, and insert into the flask a sample tube containing a piece of marble about the size of a split-pea and about 15 ml of diluted perchloric acid (1 + 1). Stopper the flask with the rubber bung carrying a delivery tube, place on a hot-plate, and boil the solution gently for 20 ± 3 minutes. The volume should not be reduced by more than about 5 ml during the boiling. Without interrupting the boiling, dip the end of the delivery tube into about 15 ml of 20 per cent. sodium carbonate solution in a test-tube, and cool in running water.

(*f*) When cool, remove the delivery tube, wash down the sides of the flask, and decant the solution into a 500-ml conical (Phillips) beaker. Do not allow the lead to enter the beaker. Wash the lead with three or four approximately 10-ml portions of 1 per cent. perchloric acid solution, and decant the washings into the beaker. Proceed to the next step without delay.

(*g*) Add 10 to 20 ml of ferric perchlorate solution (*i.e.*, sufficient to provide an excess), set aside for 30 seconds to oxidise the uranium, and then add 15 ml of diluted phosphoric acid (1 + 1). Add 120 to 150 ml of water and 5 drops of 0·3 per cent. sodium diphenylamine sulphonate indicator solution, and titrate with 0·05 *N* potassium dichromate to the violet end-point. The dichromate solution should be added until the colour reaches its full intensity. Subtract 0·08 ml of potassium dichromate solution from the titre for the indicator blank.

NOTE—If the interfering elements are removed by an ether extraction[10,11] instead of by cupferron precipitation, proceed as follows—boil off the ether, add 20 ml of perchloric acid, and continue as described in paragraph (*d*).

RESULTS

A series of uranium oxide samples from South African ore-processing plants was analysed by the proposed volumetric method. All the results obtained are shown in Table III.

TABLE III

COMPARISON OF RESULTS BY THE PROPOSED AND GRAVIMETRIC METHODS

Sample No.	Uranium found by gravimetric method,[13] %	Uranium found by proposed method: Cupferron precipitation, %	Uranium found by proposed method: Cellulose-column separation,[10,11] %	Average, %	Difference from gravimetric method, %
1	91·22	91·34, 91·53, 91·48		91·45	+0·23
2	92·74	92·52, 92·46		92·49	−0·25
3	92·92	92·84, 92·70, 92·26, 92·85		92·66	−0·26
4	93·19	93·04, 93·30		93·17	−0·02
5	93·06	93·04, 92·98		93·01	−0·05
5	93·06		92·85, 92·84, 93·06, 92·95, 92·92	92·92	−0·14
6	92·93	93·18, 93·22		93·20	+0·27
7	92·62	92·94, 92·87		92·90	+0·28

CONCLUSIONS

The results by the proposed method in Table III are in fair agreement with those by the gravimetric method,[13] which is a modification of Ryan's method.[14] Compared with other volumetric methods, the proposed method is rapid and convenient and the number of interfering elements is small. However, it is not claimed that the precision is greater than that of the gravimetric method, as an advantage of the latter is the large amount of sample that can be taken for the assay.

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The Determination of Copper and Lead in Indium Arsenide

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A method is described for determining trace amounts of copper and lead in zone-refined indium arsenide by square-wave polarography. Concentrations as low as 0·1 p.p.m. of copper and 0·2 p.p.m. of lead in a 0·5-g sample of indium arsenide can be determined.

Copper and lead are trace impurities of interest in zone-refined indium arsenide, a group III–V semiconductor material. It was thought[1] that copper might be the cause of some of the changes in indium arsenide observed after heat treatment and that lead was a probable impurity, since it was used in the purification of arsenic. A method was required for determining the concentrations of these two impurities in various sections of a zone-refined ingot of indium arsenide, and it was considered that a square-wave polarograph would be a suitable instrument for such a method because of its high sensitivity.[2] It was found that indium arsenide was soluble in a mixture of nitric and hydrochloric acids and that the residue obtained when the resulting solution was evaporated to dryness was readily soluble in 1 *M* orthophosphoric acid. In the latter solution polarographic waves caused by the reduction of copper and lead ions were obtainable.[3]

Experimental

A major problem in trace analysis by a physico-chemical method is the magnitude of the reagent-blank value. In this work blank values were minimised by using transistory grades of nitric and hydrochloric acids obtained from the British Drug Houses Ltd. These acids had extremely low copper and lead impurity concentrations (about 0·01 p.p.m.). It was found that up to about 0·5 g of indium arsenide would dissolve in a mixture of 1 ml of concentrated hydrochloric acid and 5 ml of diluted nitric acid (1 + 4). Since the copper and lead blank values for these volumes of acids were small, they were usually calculated from results found by using five times the volume required for an analysis. It was thus found that the copper and lead blank values were 0·03 and 0·01 μg, respectively.

The 1 *M* orthophosphoric acid prepared by diluting analytical-reagent grade concentrated acid had too high a concentration of copper and lead impurity to be used without prior purification, which was achieved by an ion-exchange method involving a column of Amberlite IR-120(H) resin. After purification the copper concentration was 0·005 μg per ml; the lead concentration was undetectable and was calculated to be less than 0·003 μg per ml. Since 4 ml of 1 *M* orthophosphoric acid were used in the analysis of indium arsenide, the blank value for copper was 0·02 μg; the blank value for lead was regarded as zero. The total copper and lead blank values for the analysis of indium arsenide were therefore 0·05 and 0·01 μg, respectively.

Two possible and opposing errors in trace analysis are contamination and incomplete recovery of the trace element during the analytical procedure. Chance contamination from

the atmosphere was reduced by protecting the sample and standard solutions from atmospheric dust. Solution of the indium arsenide sample and evaporation of the resulting solution to dryness were carried out in a fume cupboard fitted with a fibre-glass filter in the air inlet and an extraction fan. It was possible that the surface of the sample was contaminated, since it was cut from portions of the indium arsenide ingot on a bauxilite abrasive rubber-bonded wheel. The sample for analysis was therefore chemically etched to give a mirror finish and then thoroughly washed with distilled water.

Chance contamination from the apparatus was reduced by restricting the amount required to the minimum. For an analysis, a polytetrafluoroethylene beaker and a Pyrex-glass polarographic cell were required; before use, these were boiled for 1 hour in 1 *M* nitric acid, thoroughly rinsed with tap-water and then with distilled water and dried in air, protected from dust. Each pipette required was reserved during the series of experiments for the addition of a particular reagent.

Contamination from the apparatus may also occur if the material from which the apparatus is made or impurities in this material are dissolved by the reagents used. Polytetrafluoroethylene is reported[4] to be unattacked by any acid mixture, and tests carried out on beakers manufactured by Siemens Edison Swan Ltd. showed that there was no appreciable change in weight (of the order of 1 mg for a 50-g beaker) when they were heated at 90° C for 1 hour, each beaker containing a mixture of equal volumes of nitric and hydrochloric acids. Further, in the experiments on the recovery of copper and lead (see p. 64), the results for copper when polytetrafluoroethylene beakers were used agree with those when synthetic fused-silica crucibles were used. Synthetic fused silica, manufactured by Thermal Syndicate Ltd., is reported to contain 0·0002 p.p.m. of copper.

Incomplete recovery of copper and lead might be caused by volatilisation while the solution of indium arsenide in nitric and hydrochloric acids is being evaporated to dryness or by retention of the copper and lead on the beaker. This error was studied by adding known weights of copper and lead, as standard solutions, to the mixture of acids, evaporating the solution to dryness and determining the weights of copper and lead recovered when the residue was dissolved in 1 *M* orthophosphoric acid. The maximum temperature at which solutions were evaporated to dryness, by infra-red lamps, was 89° C. It was considered that the lower the temperature of evaporation the less the likelihood that copper and lead would be volatilised.

PREPARATION OF CALIBRATION GRAPHS—

A 4-ml portion of 1 *M* orthophosphoric acid was placed, by pipette, in a polarographic cell, and the required weights of copper and lead, as standard solutions containing 10 or 1 μg per ml, were added from calibrated 1-ml pipettes. A continuous square-wave polarogram was then recorded at a suitable sensitivity setting. The peak heights for each weight of copper and lead added were measured and corrected for volume changes, and calibration graphs were plotted. The results, measured at half the maximum sensitivity, were as follows—

Amount of copper or lead added, μg ..	0·2	0·5	1·0	2·0
Peak height of copper wave, inches ..	4·0	10·6	20·2	40·0
Peak height of lead wave, inches	1·6	3·6	7·4	14·6

The results for copper are corrected for copper present as impurity in the 1 *M* orthophosphoric acid base electrolyte. The half-wave potentials for copper and lead were —0·30 and —0·70 volt, respectively. It can be seen that both calibration graphs were linear.

DETERMINATION OF BLANK VALUES—

A 1-ml portion of a solution containing 10 mg of sodium carbonate per ml and the required volumes of diluted nitric and concentrated hydrochloric acids were placed, by pipette, in a clean 100-ml polytetrafluoroethylene beaker. (The sodium carbonate solution was added in order to facilitate solution of the residue after evaporation.) The solution was evaporated to dryness under an infra-red lamp, and the residue was dissolved in 4 ml of 1 *M* orthophosphoric acid. This solution was transferred to a polarographic cell, and a continuous square-wave polarogram was recorded at a suitable sensitivity. The peak heights for the copper and lead waves were measured, and the corresponding weights of these elements present were determined from the calibration graphs. The results are shown in Table I, in which the figures for copper have been corrected for the copper present in the 1 *M* orthophosphoric acid.

TABLE I

AMOUNTS OF COPPER AND LEAD FOUND IN NITRIC - HYDROCHLORIC ACID MIXTURE

Composition of acid mixture evaporated		Amount of copper found, µg	Amount of lead found, µg
Diluted nitric acid (1 + 4) ml	Hydrochloric acid, sp.gr. 1·18, ml		
25	5	0·16	0·05
		0·13	0·05
		0·12	0·05
		0·11	0·05
5	1	>0·02	<0·02

RECOVERY OF COPPER AND LEAD—

The procedure used to determine the recoveries of copper and lead was similar to that described for determining the blank values, but the required weights of copper and lead, as standard solutions, were placed in the cell from calibrated 1-ml pipettes before the mixture of acids was added. The residue after evaporation to dryness was dissolved in 4 ml of 1 *M* orthophosphoric acid, and a continuous square-wave polarogram was recorded for this solution. The peak heights of the copper and lead waves were measured, and the weights of each element present were found from the calibration graphs. These weights were corerected for the total copper and lead blank values; the results are shown in Table II.

TABLE II

RECOVERY OF ADDED COPPER AND LEAD

Composition of acid mixture evaporated		Amount of copper added, µg	Amount of copper found, µg	Amount of lead added, µg	Amount of lead found, µg
Diluted nitric acid (1 + 4), ml	Hydrochloric acid, sp.gr. 1·18, ml				
Evaporation in polytetrafluoroethylene beaker—					
25	5	2·00	1·94	2·00	1·95
		1·00	0·92	1·00	0·99
		0·50	0·47	—	—
		0·50	0·48	0·50	0·44
		0·20	0·17	—	—
		0·20	0·17	0·20	0·18
5	1	0·20	0·20	0·20	0·21
		0·10	0·10	0·10	0·10
		0·10	0·13	0·10	0·11
		0·05	0·07	0·05	0·07
		0·05	0·05	0·05	0·07
		0·05	0·03	0·05	0·07
		0·05	0·05	—	—
		0·05	0·05	0·10	0·12
Evaporation in synthetic fused-silica crucible—					
25	5	0·50	0·52	0·50	0·45
		0·20	0·23	0·20	0·18

METHOD

APPARATUS—

Continuous square-wave polargrams were recorded with a Mervyn - Harwell square-wave polarograph; this type of polarogram was recorded because small variations in drop-time were found to have a negligible effect on the peak heights observed. The dropping-mercury electrode and the polarograph thermostat used were made by Cambridge Instruments Ltd. The drop-time was normally 3·5 seconds and the thermostat bath was maintained at 25° C. Half-wave potentials were measured against the mercury-pool anode in the polarographic cell. Before polarography, solutions were de-oxygenated by bubbling oxygen-free nitrogen containing less than 10 p.p.m. of oxygen, by volume, through them (the nitrogen was obtained from the British Oxygen Co. Ltd.).

REAGENTS—

Unless otherwise stated, the acids used were of transitory grade, obtained from the British Drug Houses Ltd. The distilled water used contained less than 0·001 μg of copper and lead per ml. Prepared solutions were stored in polythene bottles fitted with polythene screw-on caps; no change in any solution was observed after storage for about 1 month.

Hydrochloric acid, sp.gr. 1·18.

Nitric acid, sp.gr. 1·42.

Nitric acid, diluted (1 + 4).

Hydrofluoric acid, 40 *per cent. w/v.*

Glacial acetic acid.

Orthophosphoric acid, 1·0 M—Dilute 70 ml of Judactan W.A.B.A. orthophosphoric acid, sp.gr. 1·75, to 1 litre with distilled water. Purify the solution by passing it through a 6-inch × 1-inch column of Amberlite IR-120(H) resin.

Sodium carbonate solution, 10 *mg per ml*—Prepare from Judactan W.A.B.A., sodium carbonate. The batch used was reported to contain 0·2 p.p.m. of copper and 0·3 p.p.m. of lead as impurities.

Standard copper solutions—Prepare a solution containing 1 mg of copper per ml by dissolving 3·93 g of analytical-reagent grade copper sulphate in distilled water, adding 50 ml of concentrated sulphuric acid and diluting to 1 litre with distilled water. Dilute 10 ml of this solution to 1 litre with dilute sulphuric acid (1 + 20).

1 ml ≡ 10 μg of copper.

Dilute 10 ml of this solution to 100 ml with water.

1 ml ≡ 1 μg of copper.

(Judactan W.A.B.A. sulphuric acid was used; this was found to contain less than 0·1 p.p.m. of copper and lead.)

Standard lead solutions—Prepare a solution containing 1 mg of lead per ml by dissolving 1·60 g of analytical-reagent grade lead nitrate in distilled water, adding 50 ml of nitric acid, sp.gr. 1·42, and diluting to 1 litre with distilled water. Dilute 10 ml of this solution to 1 litre with dilute nitric acid (1 + 20).

1 ml ≡ 10 μg of lead.

Dilute 10 ml of this solution to 100 ml with water.

1 ml ≡ 1 μg of lead.

(Transistory grade nitric acid was used; this was found to contain less than 0·01 p.p.m. of copper and lead.)

PROCEDURE—

A sample was cut on a cutting wheel from a portion of a zone-refined ingot of indium arsenide, and any wax present was removed by a suitable organic solvent (wax was used to mount the portion of the ingot on the cutting wheel). The sample was etched to a mirror surface in a mixture of 8 ml of concentrated nitric acid, 1 ml of glacial acetic acid and 1 ml of 40 per cent. hydrochloric acid. It was then rinsed, washed several times with distilled water and dried in air, protected from dust. The sample was then weighed, placed in a clean polytetrafluoroethylene beaker and rinsed with distilled water. By pipette, 5 ml of diluted nitric acid (1 + 4) and 1 ml of hydrochloric acid, sp.gr. 1·18, were placed in the beaker. (It was unnecessary to add 10 mg of sodium carbonate to this solution, as the residue obtained after subsequent evaporation was clearly visible.) A polytetrafluoroethylene cover was placed on the beaker, which was then heated to about 76° C on a porcelain warming-plate in a fume cupboard. Under these conditions a 0·5-g sample of indium arsenide dissolved within 30 minutes. When the sample had dissolved, the cover was removed, the beaker was placed under an infra-red lamp, and the solution was evaporated to dryness. (It took about 90 minutes to obtain a dry residue.) The residue was dissolved in 4 ml of 1 *M* orthophosphoric acid, added by pipette, the solution was transferred as completely as possible to a polarographic cell, and a continuous square-wave polarogram was recorded at a convenient sensitivity. Since the polytetrafluoroethylene beaker was not wetted by the solution it was possible to transfer the latter to the cell with little loss; experiments showed that the weight of solution remaining in the beaker after transfer was less than 0·02 g.

The determination was completed by a standard-addition method in which 0·5-, 1- or 2-μg amounts of copper and lead, as standard solutions, were added to the solution in the polarographic cell, and a continuous square-wave polarogram was then recorded. From measurements of the peak heights of the copper and the lead waves, the weights of copper and lead in the sample were calculated. It was found that the peak heights for a given concentration of copper and lead varied with the weight of sample taken.

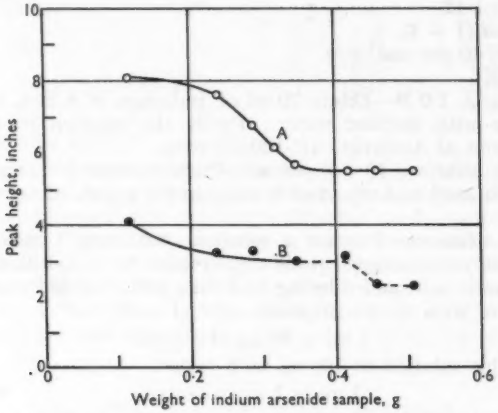

Fig. 1. Graph showing variation in copper and lead sensitivities with weight of indium arsenide sample: curve A, copper; curve B, lead. Peak heights measured at one-quarter maximum sensitivity for a concentration of 0·25 μg of copper or lead per ml

These variations in peak heights, shown in Fig. 1, were reproducible to within 10 per cent., except on occasion for lead when the weight of the indium arsenide sample was greater than 0·35 g.

Results

Some results for the determination of copper and lead in indium arsenide are shown in Table III. Fig. 2 shows the shape of a typical polarogram for a sample of indium arsenide

Table III

Amounts of copper and lead found in zone-refined indium arsenide

Results in brackets are below the recommended level of detection

Ingot	Description of sample	Weight of sample taken, g	Weight of copper found, μg	Copper content, p.p.m. w/w	Weight of lead found, μg	Lead content, p.p.m. w/w
A	All cut from same portion of ingot	0·1175	0·11	0·9	Not determined	—
		0·2203	0·20	0·9		
		0·3047	0·29	0·9		
		0·4152	0·42	1·0		
		0·5256	0·24	0·4		
B	Cut from near pure end of ingot	0·4568	0·05	0·1	Undetected	—
	Cut from near impure end of ingot	0·4800	0·30	0·6	0·12	0·3
	Cut from extreme tip of impure end of ingot	0·2536	2·56	9·9	10·3	40·4
C	Cut from near pure end of ingot	0·3659	(0·02)	(<0·1)	Not determined	—
	Cut from middle of ingot	0·3536	0·13	0·4		
	Cut from near impure end of ingot	0·2412	0·08*	0·3*		
D	All cut from same portion of ingot	0·1227	Not determined	—	(0·03)	(0·2)
		0·2285			(0·02)	(0·1)
		0·3171			(0·02)	(<0·1)

* See Fig. 2.

and Fig. 3 shows the polarogram recorded when 1 μg each of copper and lead were added to the same solution. In Fig. 2, the wave at −0·20 volt is caused by 0·13 μg of copper and the small inflexion at −0·44 volt is caused by about 0·01 μg of lead. It is evident that these half-wave potentials are different to those found for copper and lead in 1 *M* orthophosphoric acid alone and are similar to the values found in a chloride base electrolyte. It must be presumed that there is sufficient chloride present in the residue obtained before solution in 1 *M* orthophosphoric acid to cause this effect. It can be seen in both Fig. 2 and Fig. 3

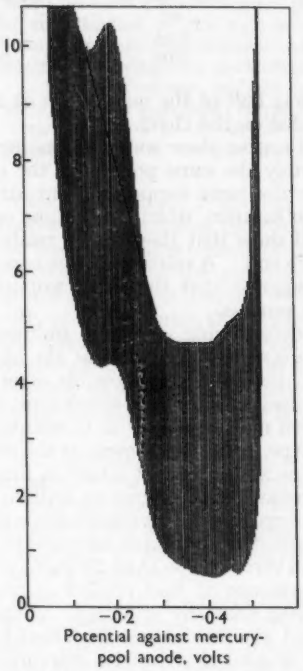

Fig. 2. Continuous square-wave polarogram at one-half maximum sensitivity for the 0·2412-g sample from indium arsenide ingot C (see Table III

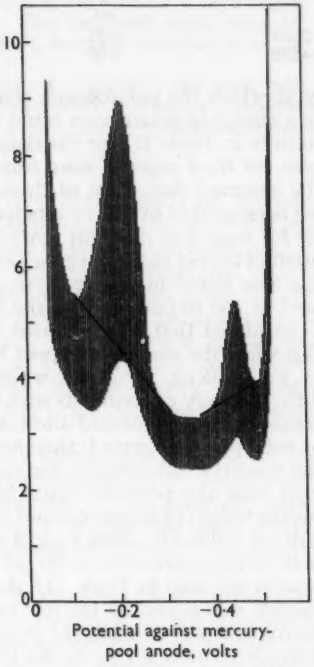

Fig. 3. Continuous square-wave polarogram at one-eighth maximum sensitivity after adding 1μg each of copper and lead to the solution that gave the polarogram shown in Fig. 2

that, after about −0·5 volt, the pen of the recorder goes off the scale. This is the beginning of the reduction wave for indium, which interferes to some extent with the preceding lead wave. The interference increases as the concentration of indium is increased and makes it more difficult to measure the peak height of the lead wave. This difficulty may explain the lack or reproducibility in the peak-height variation for lead when weights of sample greater than 0·35 g are used.

The results for the determination of copper and lead added to two samples of indium arsenide are shown in Table IV.

Discussion of results

The results in Tables I and II show that, in general, the determination of copper and lead blank values gave consistent results and that recoveries of copper and lead added to the nitric - hydrochloric acid mixtures were satisfactory. For the lowest weights of copper and lead added in the recovery experiments, it can be seen from the results on p. 63 that the peak heights were low at half the maximum sensitivity; errors of about 0·1 inch in measuring these peak heights would therefore have relatively large effects. In this work, the highest

Table IV

Recovery of added copper and lead from zone-refined indium arsenide

Samples were cut from near a portion of the ingot found to contain about 0·2 p.p.m. of copper and lead. To each sample were added 1-μg amounts each of copper and lead

Weight of sample, g	Estimated total weight of copper present, μg	Weight of copper found, μg	Estimated total weight of lead present, μg	Weight of lead found, μg
0·2957	1·07	0·94	1·05	0·79
0·4612	1·09	1·06	1·08	0·83

sensitivity at which the polarograph could be used was half of the maximum; at maximum sensitivity a complete polarogram could not be recorded on the chart.

The results in Table III for the determination of copper show some interesting features. The samples cut from ingot A were from approximately the same portion of the ingot, and it might be assumed that some of them would have the same copper concentration. This assumption is supported by the results for the first four samples, which are in close agreement. The results for copper in different portions of ingot B show that there was a relatively large concentration of copper in the extreme tip of the impure end. A relatively large concentration of lead was also found in this extreme tip, which suggests that these two impurities were concentrated at the impure end by the zone-refining process.

It is considered that the proposed method for determining copper in indium arsenide is applicable when the weight of copper in the sample is at least as large as the blank value for copper, *i.e.*, 0·05 μg. For this weight of copper, however, the probable error is large, since from the recovery experiments with 0·05 μg of copper an error of ±40 per cent. is possible and the accuracy of the standard-addition method used may be as low as to within ±10 per cent. The total probable error is therefore about ±50 per cent. However, as the percentage error in the recovery experiments for copper decreases as the weight added is increased, it is considered that the probable error for the determination of copper in indium arsenide decreases as the weight of copper present in the sample increases. This conclusion is supported by the results in Table IV; when 1 μg of copper was added to an indium arsenide sample, the recovery was at least greater than 80 per cent., *i.e.*, an error of less than 20 per cent.

The results for lead in Table III show that the amount of lead present in zone-refined indium arsenide was generally too low to be determined with any accuracy. Although the blank value for lead was 0·01 μg, the sensitivity for lead was much lower than that for copper and it is considered that 0·1 μg is the lowest weight of lead that can be determined. The probable error at this level, arrived at by considerations similar to those just discussed for copper, is about 50 per cent. The results in Table IV show that the probable error is decreased to about 20 per cent. when larger weights of lead, *e.g.*, 1 μg, are present in the sample.

Conclusions

It is considered that 0·05 μg of copper can be determined in 0·5 g of indium arsenide, *i.e.*, a copper concentration of 0·1 p.p.m. Since it is not recommended that weights of sample greater than 0·35 g of indium arsenide should be used in the determination of lead and 0·1μg of lead can be determined, the lowest lead concentration determinable is about 0·2 p.p.m.

I am grateful to Miss M. Nicholson for her assistance in the experimental work, and I acknowledge permission from the Admiralty to publish this paper.

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A Potentiostat for Electro-gravimetric Analysis

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In the instrument described the "error voltage" is fed into a d.c. amplifier based on two cathode-coupled pairs, thus avoiding the need for a high degree of stabilisation of the high-tension supply. The amplified signal operates a relay system controlling a motor that alters a variable transformer in the circuit supplying the electrolysis current.

Special features include (*i*) a sensitivity of $\pm 2{\cdot}5$ mV, (*ii*) high zero stability, (*iii*) high input impedance, (*iv*) the relay-controlled motor is stopped "dead" as soon as the signal ceases and (*v*) a high degree of smoothing and discrimination in the electrolysis supply circuit.

It has been known for many years that a marked increase in the selectivity of electro-analysis can be obtained by controlling the cathode (or anode) potential. No great use was made of the method, however, while control of the potential had to be obtained by manual adjustment of the voltage applied to the electrolytic cell; the process is tedious and time-consuming and the control achieved is inexact. With the appearance of devices to control the potential automatically (potentiostats), the method has become more popular, particularly in the U.S.A., where about a dozen instruments have been described. Some of these are simple in design, but of limited application; however, in the main they are based on fairly complicated electronic circuits. Details of a few circuits have been published in this country, but it is fairly true to say that the method has not been applied here to any extent because of the lack of suitable instruments.

Most, but not all, potentiostats are based on the following principles: (*a*) the e.m.f. of the cell formed by the working electrode and the reference electrode is connected in series-opposition with the required e.m.f. set on a potentiometer, (*b*) the resulting "error voltage" is amplified and (*c*) the amplified signal is used to actuate a motor that alters a variable transformer or resistance in the circuit supplying the electrolysis current and thus alters the voltage applied to the electrolytic cell.

Two main systems of amplification are used. In the first, the error voltage is fed into a converter, where it is chopped into a.c. This is then amplified, usually to power level, when the amplified signal is fed to a reversible two-phase motor arranged so that the direction of rotation of the motor is governed by the sign of the error voltage. This sytem is particularly popular in the U.S.A., where the Brown Elektronik indicating potentiometer has been used directly with little modification. It is also the basis of the instrument described by Palmer and Vogel,[1] who made the desirable addition of placing a cathode-coupled pair before the converter, thus increasing the input resistance. Alternating, current amplification has the advantage that it does not require stabilisation of the voltage of the power supplies; on the other hand, instruments based on the above-mentioned system tend to be bulky and less readily adaptable for other purposes. The possibility of amplifying the a.c. to a lower level and applying the amplified signal to the grids of a phase-discriminating double triode with relays in the anode leads does not appear to have been exploited.

Alternatively, the error voltage is fed into a d.c. amplifier and the output is used to operate relays controlling the rotation of the motor. If a simple direct-coupled d.c. amplifier is used, the voltage of the high-tension and heater supplies must be stabilised. Consequently, the power supplies are somewhat complicated or, if partly or wholly derived from batteries, inconvenient. Chambers[2] appears to be alone in using the more stable push-pull type of d.c. amplifier. He used one stage of amplification followed by a galvanometer relay of unstated sensitivity. The instrument described here is in some respects an extension of this. The galvanometer relay has been replaced by a less sensitive but more rugged relay, the sensitivity of the instrument increased and a number of improvements have been introduced.

In designing the instrument described, we were guided by the criteria stated by Milner and Whittem[3] and also by the desire to make the instrument as versatile as possible without undue complication. The general plan of the instrument is shown in Fig. 1.

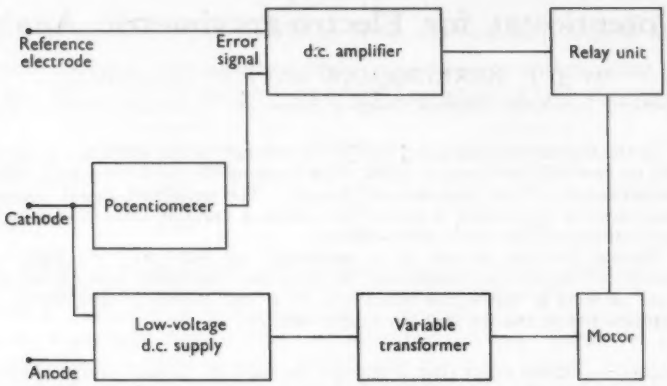

Fig. 1. General plan of instrument

POTENTIOMETER—

This is a Cambridge portable type, modified so that it can be standardised without interruption of its e.m.f. signal. It can be replaced if desired by a potentiometer constructed in the laboratory, provided that it is of high quality. Several instruments described in the literature contain instead a potential-divider circuit and voltmeter; in our opinion this is barely sufficient for the best work.

Some instruments incorporate a valve voltmeter to measure the cathode potential independently of the controlling circuit. This introduces a further and unnecessary complication because a valve voltmeter is not a particularly accurate method of measuring an e.m.f. In the instrument described any deviation greater than 2·5 mV in the cathode potential from the prescribed value is indicated by the relays. If further information is required, a milliammeter may be placed in series with the moving-coil relay, RL_1. The meter can be calibrated for given error inputs, and thus a fairly accurate measure of the deviation in the cathode potential can be obtained without impairing the sensitivity of the instrument.

D.C. AMPLIFIER—

For this use is made of the cathode-coupled pair, which comprises two valves with a resistor in the common cathode lead. The signal is fed to the grid of one valve and the grid of the second valve is held at a fixed potential. The output is drawn from across the anodes. The use of a push - pull circuit and the negative feedback provided by the cathode resistor gives considerable stability, but reduces the gain. The system is much less sensitive to variations in the high-tension voltage than is a simple direct-coupled amplifier, and so quite simple stabilisation of the high-tension voltage (gas-discharge tubes) is effective. Stabilisation of the heater supplies was found to be unnecessary.

Two such stages of amplification are used (see Fig. 2). In the first, the error-voltage input is connected to one grid of a double triode. In order to obtain sufficient amplification at this stage it is necessary to raise the voltage of the signals to the grids. (This is done by means of a 15-volt battery, which supplies negligible current and therefore has very long life.) The resistance in the anode leads is large in this circuit so as to obtain good voltage amplification; for a high-resistance load the voltage amplification obtained in this stage is 20. The output from the double triode is connected to the grids of two selected* output valves (KT61, triode connected). The output from this stage is connected across the moving-coil relay.

The amplifier is balanced by operating switch S_1, so as to impress the same voltage on both grids of the double triode, and adjusting resistor R_8 until RL_1 is open. The drift

* This selection is not critical and consists in choosing two valves not markedly different; usually the first two tried will be satisfactory. We found no evidence for the need to select the double triode valve in the first stage; four 6SN7 valves were tried and all gave substantially the same performance.

of this zero setting was never found to correspond to a change in the error signal of more than 1 mV in 3 hours, *i.e.*, it is negligible for all practical purposes.

The maximum current drawn from the reference electrode is 10^{-8} amp.

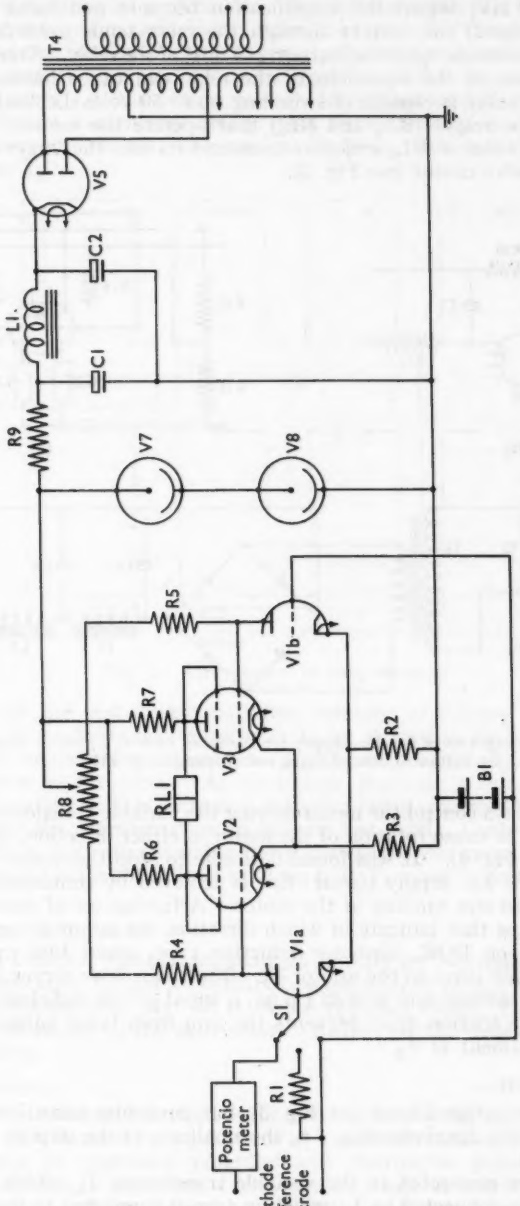

Fig. 2. Circuit diagram of d.c. amplifier (for values of components, see Appendix, p. 74)

RELAYS AND MOTOR—

The moving-coil relay RL_1 has a resistance of 700 ohms and a minimum operating current of 125 μA when the two coils are connected in series. Positive action of the relay is obtained with an input to the amplifier of 2·5 mV. The design of the amplifier is such that at high ($\sim$ 100 mV) inputs the amplification becomes non-linear and at still higher inputs (1 volt or greater) the current through the relay tends towards a constant value, which is below the maximum operating current (10 mA) of the relay. Consequently, whatever the state of unbalance of the input circuit the relay cannot be harmed.

The moving-coil relay is capable of switching up to 50 volts d.c. and thus of controlling directly the P.O. type relays (RL_2 and RL_3) that operate the motor. However, in order to obtain better operation of RL_1 and also to extend its life, the larger relays are operated via a conventional valve circuit (see Fig. 3).

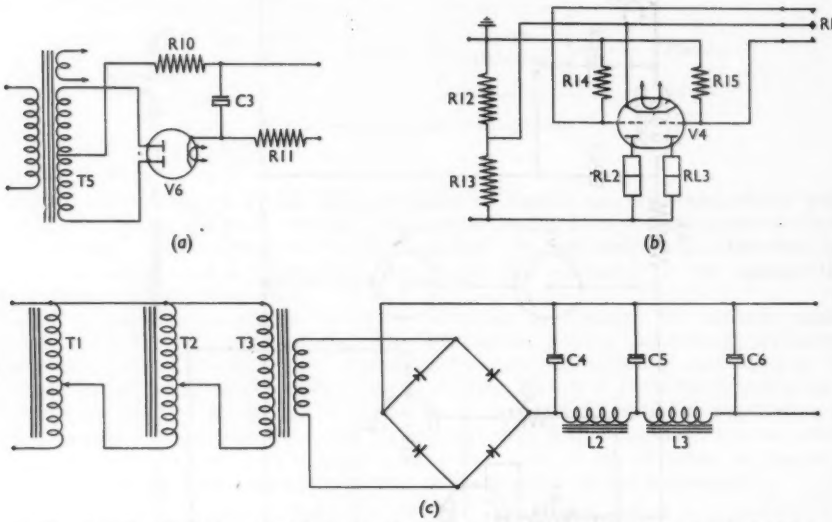

Fig. 3. Circuit diagrams of (*a*) d.c. supply for stopping motor, (*b*) valve relay and (*c*) low-voltage d.c. supply (for values of components, see Appendix, p. 74)

The two larger relays control the motor driving the variable transformer T_2, the contacts being connected so as to cause rotation of the motor in either direction, depending on which relay is operated (see Fig. 4). It was found desirable to stop the motor "dead" as soon as either relay opened the a.c. supply circuit; this is achieved by simultaneously switching in a 50-volt d.c. supply to one winding of the motor. A further set of contacts on the relays is used to control lamps that indicate in which direction the motor is turning.

The motor (Drayton RQR, capacitor-induction type, speed 1·09 r.p.m.) is connected via a chain and a friction drive to the arm of T_2. The chain drive serves to reduce the speed of rotation of the transformer arm to 0·33 r.p.m., a speed giving sufficiently rapid correction without hunting. The friction drive prevents the arm from being turned too far and also permits manual adjustment of T_2.

LOW-VOLTAGE D.C. UNIT—

This is of the conventional type (see Fig. 3), but particular attention has been paid to the smoothing and to the discrimination, *i.e.*, the smallness of the step by which the applied voltage can be altered.

The a.c. mains are connected to the variable transformer T_1, which is hand-operated. The output from this is connected to T_2, which in turn is connected to the step-down transformer T_3. Then with T_2 at its maximum setting, the voltage supplied by T_3 is continuously variable between 0 and the rated output of T_3 by adjustment of T_1.

The a.c. from T_3 is rectified by a bridge selenium rectifier and the resulting output is smoothed by the circuit shown. Most of the previously described instruments incorporate only a single-section filter with a much smaller choke; tests on such circuits showed that at currents of the order of 1 amp the ripple was far too large. With the circuit described here, tests made in typical electrolyses showed that, for a cell resistance of 1 ohm, the ripple in the e.m.f. of the cell formed by the cathode and reference electrode did not exceed 3 mV (r.m.s.) at a current of 2·5 amps; at lower currents the ripple was considerably less.

Whatever the smoothing circuit used, d.c. supplies of this type show poor regulation over their working range, *i.e.*, the output voltage drops considerably as the current taken is increased. This goes to worsen the already poor discrimination shown by circuits incorporating a step-down transformer with various tappings, but not including a regulating variable transformer T_1.

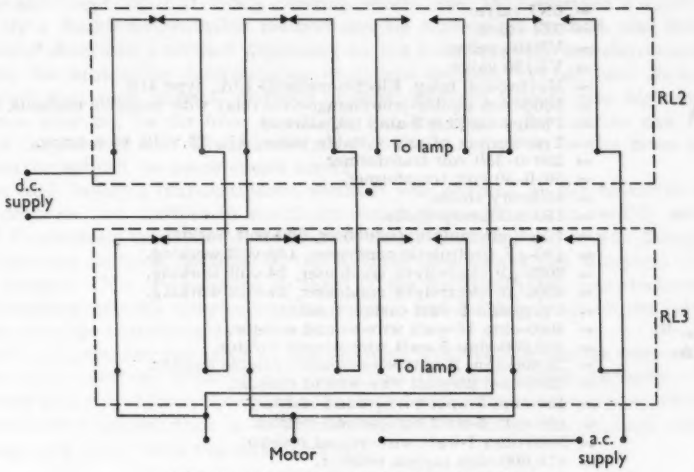

Fig. 4. Arrangement of relay contacts

With our circuit the best discrimination is obtained as follows. At the beginning of the electrolysis, T_2 is set at its maximum with T_1 set at zero so that the motor drives in a direction that would increase the output from T_2. The output from T_1 is then slowly increased until the motor reverses direction. As electrolysis proceeds, a smaller applied voltage is required and so the output of T_2 is decreased, the effect being enhanced by the poor regulation; the latter also causes the discrimination to become less good. Consequently it is desirable at some suitable stage, *e.g.*, after washing down the electrolysis vessel when the current has fallen considerably, to reduce the output of T_1 so that T_2 again operates near its maximum output.

OPERATION OF THE INSTRUMENT—

Switch on the amplifier circuit, and allow 15 minutes for "warming up." Then balance the amplifier by operating S_1 and adjusting R_8 until neither of the indicating lamps is on. Standardise the potentiometer, and set it to the required value. Connect the electrolysis cell and reference electrode to the instrument. Switch on the remaining circuits. Adjust T_1 as indicated above.

OTHER APPLICATIONS—

Although the instrument described is intended primarily for use in electro-gravimetric procedures, it can also be used for separations at a mercury cathode at controlled potential, coulometric analysis at controlled potential and electrolytic preparations at controlled potential. The amplifier is such that a relay can be made to operate at a given potential or small current input, and therefore this part of the instrument can be used in automatic potentiometric apparatus, in automatic coulometric titrations with an amperometric, potentiometric or photometric end-point, etc.

Conclusion

We are well aware of improvements that could be made to the instrument described, *e.g.*, the use of newer valves in the amplifier. However, the instrument has been thoroughly tested and has given good service over the past 3 years when it has been used by a number of operators for all of the above-mentioned purposes.

APPENDIX

List of components used in the construction of the instrument (Figs. 2 and 3)

V_1	= 6SN7 valve.
V_2, V_3	= KT61 valve.
V_4	= 6N7 valve.
V_5, V_6	= 5Z4 valve.
V_7	= VR105 valve.
V_8	= VR150 valve.
RL_1	= Moving-coil relay, Electro-methods Ltd., type 416.
RL_2, RL_3	= 5000-ohm double-pole change-over relay with tungsten contacts, P.O. type.
T_1, T_2	= Philips variable 2-amp transformer.
T_3	= Transformer of any suitable value, *e.g.*, 15 volts at 5 amps.
T_4	= 350–0–350 volt transformer.
T_5	= 50–0–50 volt transformer.
L_1	= 40-henry choke.
L_2, L_3	= 150-millihenry choke.
C_1, C_2	= 16-μF electrolytic condenser, 500-volt working.
C_3	= 400-μF electrolytic condenser, 150-volt working.
C_4, C_5	= 2000-μF electrolytic condenser, 24-volt working.
C_6	= 4000-μF electrolytic condenser, 24-volt working.
R_1	= 1-megohm 1-watt carbon resistor.
R_2, R_3, R_6, R_7	= 5000-ohm 10-watt wire-wound resistor.
R_4, R_5, R_{13}	= 100,000-ohm 5-watt wire-wound resistor.
R_8	= 25,000-ohm 3-watt wire-wound variable resistor.
R_9	= 3300-ohm 20-watt wire-wound resistor.
R_{10}	= 450-ohm 5-watt wire-wound resistor.
R_{11}	= 100-ohm 5-watt wire-wound resistor.
R_{12}	= 5000-ohm 1-watt wire-wound resistor.
R_{14}, R_{15}	= 470,000-ohm carbon resistor.
B_1	= 15-volt battery.
S_1	= Yaxley make-before-break switch.
Miscellaneous	17-volt 5-amp selenium rectifier. 0·5-μF 350-volt working paper condensers across the contacts of the P.O. relays for spark quenching.

Not shown are switches on the a.c. supply to the amplifier, the motor and the d.c. supplies. If desired, a switch may be incorporated in the appropriate grid lead in the valve relay circuit so that the instrument can be made to control in one direction only.

The whole is housed in a standard Imhof case 24 inches × 12 inches × 15 inches (No. 1053C).

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Notes

THE DEXTROSE AND MALTOSE CONTENTS OF COMMERCIAL LIQUID GLUCOSE

THE proportions of individual sugars in liquid glucose often have a marked effect on the texture and storage life of confectionery and other food products containing it. It is therefore a disadvantage that the precise content of the individual sugars is not indicated by the conventional data—Baumé and "dextrose equivalent"—used by manufacturers.

There are many references in the literature to the dextrose and maltose contents of liquid glucose, *e.g.*, Bryant and Jones[1] obtained figures by a bakers' yeast fermentation method. Schultz, Fisher, Atkins and Frey[2] obtained widely differing results when they analysed a number of starch hydrolysates by a double-fermentation method and by a chemical method, and Fetzer, Evans and Longenecker[3] described a method depending on the initial enzymic transformation of dextrin to maltose and the subsequent determination of optical rotation. It has been shown by paper chromatography[4] that such classical methods of sugar analysis usually give high results. The dextrose figures may not be far from the truth, but the figures for maltose can be as much as twice what they should be. The fermentation methods appear to be the more reliable, but even these can be subject to considerable errors.

Patterson and Savage's carbon-column method[5] was adopted as the tentative method for determining dextrose and maltose in starch-conversion products at the twelfth session of the International Commission on Uniform Methods of Sugar Analysis, and it was thought that the dextrose and maltose contents, obtained by this method, of some samples of typical liquid glucose would be of interest. We are indebted to three of the leading British manufacturers of liquid glucose for providing us with samples for the purpose and for supplying all the information in Table I other than the carbohydrate contents.

After each analysis, the remnants of the dextrose and maltose eluates were evaporated to small volume under reduced pressure and submitted to paper chromatography. The higher saccharides were also eluted from the column by 50 per cent. ethanol and treated similarly. The paper chromatograms showed that in every instance both dextrose and maltose were pure and had been completely eluted from the carbon column.

TABLE I

DEXTROSE AND MALTOSE CONTENTS OF LIQUID GLUCOSE

Manufacturer's description of sample	° Baumé	Dextrose equivalent,* % w/w	Total sugar solids, % w/w	Dextrose found, % w/w	Maltose found, % w/w	Higher saccharides (by difference), % w/w
Samples from manufacturer A—						
Normal (43° Baumé)	43·1	46·7	81·2	16·4	11·4	53·4
Low dextrose equivalent (41·5° Baumé)	41·6	35·0	78·9	10·4	8·6	59·9
High dextrose equivalent (43° Baumé)	43·1	54·1	81·2	22·4	13·0	45·8
High dextrine (45° Baumé) ..	44·9	37·7	85·1	14·4	10·2	60·5
Samples from manufacturer B—						
Confectioners' (acid conversion) ..	43·1	39·2	82·0	14·6	10·6	56·8
Intermediate dextrose equivalent (acid conversion)	—	~55	—	29·2	14·4	—
High dextrose equivalent (acid enzyme conversion)	—	~65	82·3	33·2	24·3	24·8
Samples from manufacturer C—						
43° Baumé (acid conversion) ..	43	34	83·2	10·9	8·0	64·3
43° Baumé—standard (acid conversion)	43	42	83·2	15·4	10·6	57·2
43·5° Baumé (acid conversion) ..	43·5	55·5	84·5	27·9	13·6	43·0
Acid/enzyme converted (*ex* U.S.A.)	—	64	83·2	30·2	22·3	30·7

* Percentage by weight of reducing sugars, calculated as dextrose, on a dry basis.

We thank Miss M. Scarles for carrying out the determinations and the Acting Government Chemist for permission to publish this Note.

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THE DETERMINATION OF IRON IN TIN OF 99·999 PER CENT. PURITY

It became necessary to determine accurately the iron content of extremely pure tin in the range 0·5 to 5 p.p.m., but it was found that normal methods[1,2,3] were inadequate. In determining certain impurities in tin, advantage is taken of the fact that tin bromide is volatile and can thus be readily removed. The obvious method, therefore, for extending the range of the procedures for determining iron to cover lower ranges of iron contents would be to use larger weights of sample, but these weights require larger amounts of reagents for solution, and a point is soon reached at which the blank solution contains considerably more iron than does the sample. For example, when the iron in a 10-g sample is determined as thiocyanate and the reagents used are of the best quality available, the reagent blank is equivalent to about 10 p.p.m. of iron. To increase the accuracy of this method, therefore, purification of the reagents was essential. When this was carried out the blank value was reduced to 0·7 p.p.m. of iron.

Experimental

Purification of reagents—

The reagents used in appreciable amounts were concentrated hydrochloric and hydrobromic acids and bromine. It was found that analytical-reagent grade hydrochloric acid was almost pure enough for our purpose, containing 0·2 to 0·5 p.p.m. w/v of iron, but the hydrobromic acid and bromine would introduce substantial amounts of iron. These three reagents were at first purified by distillation, which, for hydrobromic acid, had to be carried out under reduced pressure; it was later found that hydrochloric and hydrobromic acids could be sufficiently purified by treatment with an anion-exchange resin.

Hydrochloric acid—The hydrochloric acid was passed through a 7-inch × 1½-inch column of Amberlite IRA-400 resin at the rate of about 25 ml per minute, and the first 200 ml of effluent were rejected. The resin can be regenerated after use by washing with dilute hydrochloric acid (1 + 9).

To ascertain which form of iron was most easily removed, amounts of ferrous and ferric iron equivalent to 10 p.p.m. w/v were added to portions of the hydrochloric acid, and the solutions were passed through the column. After treatment, the acid to which ferrous iron had been added contained 0·1, 0·08 and 0·09 p.p.m. w/v of iron and that to which ferric iron had been added contained 0·09, 0·08 and 0·06 p.p.m. w/v, *i.e.*, both ferrous and ferric forms were removed to the same extent.

Hydrobromic acid—The hydrobromic acid was purified by passage through the ion-exchange column described above, a glass-wool plug being used to prevent the resin from floating; as with hydrochloric acid, the first 200 ml of effluent were rejected. The resin can be regenerated after use by washing with dilute hydrobromic acid (1 + 9).

To test the purification procedure, amounts of ferrous and ferric iron equivalent to 10 p.p.m. w/v were added to portions of the hydrobromic acid, and the solutions were passed through the column. After treatment, the acid to which ferrous iron had been added contained 0·7, 0·8 and 1·5 p.p.m. w/v of iron, whereas that to which ferric iron had been added contained 0·1, 0·1 and 0·1 p.p.m. w/v of iron; iron was therefore most easily removed in the ferric state. In all subsequent purifications of hydrobromic acid, 1 drop of bromine per litre was added before passage through the column. Analytical-reagent grade hydrobromic acid was found to contain from 1·0 to 1·5 p.p.m. w/v of iron; after passage through the ion-exchange resin, this value was reduced to 0·1 p.p.m. w/v.

Bromine—Analytical-reagent grade bromine containing between 1·0 and 1·5 p.p.m. w/v of iron was purified by distillation from an all-glass apparatus, after which it contained about 0·2 p.p.m. w/v of iron.

REMOVAL OF TIN—

In most of the methods described for the removal of tin, a mixture of hydrobromic acid and bromine is used. Tin is generally removed in one evaporation, but sometimes a large residue is left. This residue is extremely difficult to remove, and several treatments with hydrobromic acid and subsequent evaporations are often needed to effect complete removal of tin. However, a mixture of hydrochloric acid, hydrobromic acid and bromine will successfully remove the tin in one evaporation.

METHOD

APPARATUS—

Clean all glassware with hot hydrochloric acid, and rinse with distilled water.

REAGENTS—

In addition to concentrated hydrochloric and hydrobromic acids and bromine, purified as described above, the reagents required are—

Hydrochloric acid, diluted (1 + 4).

Ammonium persulphate solution, 1 per cent. w/v.

Ammonium thiocyanate solution, 10 per cent. w/v.

PROCEDURE—

Prepare the sample in a convenient form by melting the tin in a flame and allowing the molten metal to drip into a large glazed-porcelain evaporating basin from a height such that thin sheets are formed from the individual drops.

Weigh 10 g of sample into a 150-ml beaker, add 20 ml of concentrated hydrochloric acid, and then add, with cooling, a mixture of 25 ml of concentrated hydrobromic acid and 15 ml of bromine. Warm gently, if necessary, to complete solution of tin, cover the beaker with a watch-glass, place on a hot-plate, and allow to evaporate. When almost dry, remove the beaker from the hot-plate, take off the watch-glass, and allow the fumes of tin to escape. (Note that the residue must not be baked at this stage otherwise it is rendered difficult to dissolve.)

Add 5 ml of diluted hydrochloric acid (1 + 4), cover with the watch-glass, and concentrate the solution to half its volume. Cool, add 0·5 ml each of 10 per cent. ammonium thiocyanate solution and 1 per cent. ammonium persulphate solution, and dilute to 10 ml with water in a calibrated flask. Measure the optical density of the solution in 1-cm cells with a Spekker absorptiometer fitted with Ilford No. 603 filters. Carry out a blank determination on the reagents, and deduct the optical-density blank value.

Prepare a calibration graph in the usual manner. The graph corresponds to the equation—

$$\text{Iron present, p.p.m.} = \text{Absorptiometer-drum difference} \times 0{\cdot}09.$$

RESULTS

The iron content of a sample was determined in triplicate by four different operators, the mean result being 0·75 p.p.m. The same sample, after the addition of the equivalent of 2 p.p.m. of iron as a solution, was again analysed in triplicate by four different operators; the mean of the results was 2·6 p.p.m. of iron. The results obtained, together with the blank values, are shown in Table I.

TABLE I

RECOVERY OF IRON BY THE PROPOSED METHOD

Each result for iron content is corrected for the mean blank value

Operator	Blank value, as iron, p.p.m.	Iron content of sample, p.p.m.	Iron content of sample *plus* 2 p.p.m. of added iron, p.p.m.
A	0·80	0·65	2·6
	0·80	0·75	2·5
	0·65	0·80	2·7
B	0·60	0·65	2·5
	0·65	0·80	2·4
	—	0·65	2·7
C	0·85	0·75	2·8
	0·75	0·65	2·6
	0·75	0·75	2·6
D	0·90	0·80	2·7
	0·80	0·90	2·3
	0·85	0·90	2·5

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Book Reviews

COLORIMETRIC METHODS OF ANALYSIS: INCLUDING PHOTOMETRIC METHODS. Volume IIA. By FOSTER DEE SNELL, Ph.D., and CORNELIA T. SNELL, Ph.D. Pp. x + 793. Princeton, N.J., New York, Toronto and London: D. Van Nostrand Co. Inc. 1959. Price $15.00; 112s. 6d.

The current third edition (1948–1954) of Snells' "Colorimetric Methods of Analysis" is a four-volume work, and this book is a supplement to Volume II (reviewed *Analyst*, 1951, **76**, 183) covering up to January, 1956. There are 68 chapters, each dealing with an element or radicle, and, in general, the details of methods are preceded by descriptive matter relating to the preliminary treatment of various materials likely to be investigated. Naturally and rightly, the plan of the original book has been maintained, with some improvement in the guidance given to the reader by way of the discussions.

The scope of the subject has been extended to cover the colour of flames, but it is perhaps questionable if this expansion is justified, because in the opinion of the reviewer the subject of flame photometry needs more extended treatment if the account of its applications is to be of practical value to those not already initiated in this subject. It may be that flame photometry has been included because, as stated, the American Society for Testing Materials has tentatively accepted the technique for the determination of sodium and potassium in cement, and the method is here given in full.

The outlook of the authors is creditably cosmopolitan, but, naturally, there is a certain bias to transcribe from American sources; notwithstanding the impressive and extremely useful coverage of the world's literature there does not appear to be any mention of the many colorimetric methods incorporated in the British Standards of the B.S.I., which would have been helpful for readers in this country and the Commonwealth.

Compared with the magnitude of the work before us these are but minor details, because, in fact, each page bears the imprint of careful and concentrated study. The book can be recommended as a useful addition to the library of every analyst.

NOEL L. ALLPORT

PRECIPITATION FROM HOMOGENEOUS SOLUTION. By LOUIS GORDON, MURRELL L. SALUTSKY and HOBART H. WILLARD. Pp. viii + 187. New York: John Wiley and Sons Inc.; London: Chapman and Hall Ltd. 1959. Price $7.50; 60s.

This book has been written by the three chemists who have contributed most to the subject of precipitation from homogeneous solution, an idea known for a long time but not used to any extent until 1937, when Professor Willard, the senior author, initiated its modern development by his work with Dr. Tang on the precipitation of aluminium ions as a basic salt by means of urea.

The essence of the method is that the precipitant should be generated slowly by a chemical reaction within the solution. The precipitate also, as a consequence, is formed slowly and in a way that often eliminates the undesirable effects frequently found in a conventional precipitation. Further, under correct conditions of formation it can have improved characteristics in uniformity of particle size, compactness, ease of filtering and washing and greater purity. The best-known example is the use of urea to generate ammonia on heating the solution and so produce a gradual increase in pH and controlled precipitation of hydrated oxides, basic salts, oxalates and the like.

The book covers the existing literature of this field and, after an introduction, deals in six chapters with the precipitation of hydrated oxides and basic salts, phosphates, oxalates, sulphates, and sulphides and with miscellaneous precipitations, many of which are novel and ingenious. Methods of generating the precipitant in solution and procedures for determining a wide range of ions, mainly cations, are detailed with appropriate references to the literature. Many of the procedures given will save the reader the trouble of going to original papers should he want to try a method for himself. Numerical results showing how precipitation from homogeneous solution can provide effective separations of one ion from another are also quoted. These separations are often so much cleaner than those obtained by conventional methods that re-precipitation becomes unnecessary.

Many analysts will want to know how some of these methods behave in the presence of the relatively high concentrations of sodium or potassium ions necessarily introduced in a solution when an alkali fusion of a sample has to replace an acid attack. Homogeneous precipitation demands a close control of conditions, and it is possible that such high concentrations of alkali ions would introduce serious difficulties in its application. The data presented in the various chapters give little help on this point, the effect of sodium or potassium ions being seldom mentioned.

Two further chapters, more theoretical than the earlier ones, deal with co-precipitation in its various aspects and with fractional precipitation, which is shown to be improved by applying the technique of precipitation from homogeneous solution, a greater yield of product of desired purity being obtained with fewer fractionations than are required by conventional methods. A final chapter describes the applications of the technique utilised in chemical technology.

The book is well-written with only a few misprints, but the practice of avoiding as many hyphens as possible leads to such odd-looking words as "preignition" and "reignite." The price asked seems a lot to pay for 187 pages of information much of which is available in the literature. Even so, the volume contains in a convenient form a great deal that will be interesting and useful to those who still believe in precipitation as an indispensable part of chemical analysis. May the authors' hope that the information contained in their book will not only lead to greater application of precipitation processes, but will also stimulate further research on precipitation phenomena, be realised.

L. S. THEOBALD

NOTES DU SERVICE DE GÉOLOGIE ET DE PROSPECTION MINIÈRE: ANALYSE DES EAUX. MÉTHODE UTILISÉE AU LABORATOIRE DU SERVICE DE GÉOLOGIE ET DE PROSPECTION MINIÈRE DE L'A.O.F. By B. MARTINET, Ing. chim. Pp. 26. Dakar, French East Africa: Haut Commissariat de la Republique en A.O.F.

The methods of analysis are designed to assist geological studies and are therefore limited mainly to the major mineral constituents. In the choice of methods particular attention has been given to simplicity and convenience in dealing with thousands of samples per annum. Most of the methods are similar to those in current British practice. The descriptions are precise and up to date.

Titration with EDTA is used for calcium and magnesium and indirectly for sulphate after adding excess of barium chloride. Some doubt might be expressed about its accuracy for magnesium or for sulphate when the amounts present are small in relation to the calcium, as they so often are in natural waters.

An interesting investigation is described of an improved complexometric-titration method for calcium and for sulphate with calcein as the indicator (compare *Analyst*, 1957, **82**, 285); unfortunately, this is not very suitable for the determination of magnesium.

R. C. HOATHER

ESSENTIAL FATTY ACIDS. Edited by H. M. SINCLAIR. Pp. xviii + 268. London: Butterworths Scientific Publications; New York: Academic Press Inc. 1958. Price 50s.; $9.50.

This book contains the Proceedings of the Fourth International Conference on Biochemical Problems of Lipids, held in Oxford in July, 1957. The first two conferences on this subject were of a rather general character, but the third, held in Brussels in 1956, dealt with a particular subject, The Blood Lipids and The Clearing Factor. The fourth conference continued this policy and discussed The Essential Fatty Acids, that is, those fatty acids of a polyunsaturated nature that have been shown to lead to a major decrease in plasma cholesterol when they form a substantial proportion of the diet.

The conference was divided into six sessions, and of these the first two dealt largely with the problems of determining polyunsaturated fatty acids. There were papers on determinations by gas chromatography, by alkali isomerisation, by the use of near-infra-red spectra and by an enzymatic method. The separation of cis-cis-linoleic acid from its geometric isomers is discussed; methods considered including one of isotopic dilution; and dialysis through a rubber membrane is suggested for the isolation of phospholipids. There are reports of work undertaken to establish the composition of fish oils, comparing methods now available with those of twenty years ago.

An excellent summary of the present position of this analytical work is given by J. F. Mead, R. T. Holman, A. T. James, P. N. Williams and J. A. Lovern. The remainder of the book, almost three-quarters of the whole, is concerned with the absorption and distribution of the polyunsaturated fatty acids in animals and their biochemical functions. K. A. WILLIAMS

Publications Received

JOURNAL OF ELECTROANALYTICAL CHEMISTRY. Volume 1, No. 1, August, 1959. Edited by G. CHARLOT, J. O'M. BOCKRIS and C. N. REILLEY. Pp. vi + 100. Amsterdam: Elsevier Publishing Company. Subscription price 125s.; $17.50; Dfl. 66.50 per volume.

A new journal.

THE OPERATION OF STERILISING AUTOCLAVES. A Symposium held at Brighton Technical College School of Pharmacy on May 9th, 1959. Pp. ii + 45. London: The Pharmaceutical Press. 1959. Price 7s. 6d.

INTERNATIONAL CONFERENCE ON CO-ORDINATION CHEMISTRY: London, April 6th–11th, 1959. Organised by the Chemical Society under the sponsorship of the International Union of Pure and Applied Chemistry. Special Publication No. 13. Pp. iv + 204. London: The Chemical Society. 1959. Price 42s.; $6.00.

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REPORT OF THE ANALYTICAL METHODS COMMITTEE: REPRINTS

The Determination of Tocopherols

THE Report prepared by the Vitamin-E Panel, "The Determination of Tocopherols in Oils, Foods and Feeding Stuffs," reprinted from *The Analyst*, June, 1959, **84**, 356–372, is now available from the Secretary, The Society for Analytical Chemistry, 14 Belgrave Square, London, S.W.1, price to members, 1s. 6d. each; to non-members, 2s. 6d. each.

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